2015

Influence of Bradyrhizobium inoculation and fungicide treatment on development and yield of selected pulse crops; Nutritional composition of pulse legume leaves and the impact of leaf removal on yield

Rosemary Bulyaba
Iowa State University

Influence of *Bradyrhizobium* inoculation and fungicide treatment on development and yield of selected pulse crops

Nutritional composition of pulse legume leaves and the impact of leaf removal on yield

by

Rosemary Bulyaba

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology

Program of Study Committee:
Andrew Lenssen, Major Professor
Allen Knapp
Gail Nonnecke
Ajay Nair

Iowa State University

Ames, Iowa

2015

DEDICATION

This thesis is dedicated to my mother, Jane Mugabi, thank you for being a great inspiration and for your unwavering love. I also appreciate and dedicate this thesis to my family, close relatives and all the amazing people especially Ivan Charles Muyanja whom God has blessed me with and used to encourage and stand by me each and every day; to my major professor, Dr. Andrew Lenssen; thank you for believing in me and for being a great role model. Lastly, I dedicate this thesis to God who has given me life, wisdom and all the wonderful people in my life to see me through.

TABLE OF CONTENTS

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to extend immense gratitude to my major professor, Dr. Andrew Lenssen for his relentless guidance, encouragement and support during the course of this Master's program. Thank you for always being patient with me, for teaching me humility, life and career skills beyond just classes and research and for being a great mentor.

I also thank my committee members, Dr. Gail Nonnecke, Dr. Allen Knapp and Dr. Ajay Nair. Thank you for your guidance, patience and flexibility to meet and discuss my research projects and at times, just to discuss my well-being and experience here at Iowa State University. Thank you.

I also extend my gratitude to the department for agronomy for financial support and educational training throughout my program, to the numerous faculty and staff at Iowa State University from whom I have gained vast knowledge through their classes and course work and others for their technical assistance and knowledge. I also appreciate my fellow office mates and friends and lastly to my family and relatives for their unconditional love.

May God bless you all and reward you abundantly.

ABSTRACT

Declining soil fertility due to inadequate nitrogen in most soils limits farmers' goals of increasing yield. There is environmental and agricultural benefit of reducing dependence on nitrogen fertilizer by emphasizing adaptation of favorable farming practices that minimize nitrogen fertilizer. Cowpea [*Vigna unguiculata* (L.) Walp.] and lablab (*Lablab purpureus* (L.)] are pulses with potential for grain, forage, and cover crops in the Midwest of United States, but little is known about their growth, development, and productivity potential in Iowa. We conducted a two-year field study to determine the influence of rhizobia inoculation and fungicide seed treatment of selected pulses. The objectives of this study were to i) determine the influence of fungicide seed treatment and inoculation on nodulation and nitrogen fixation of four pulses; soybean '92Y82', cowpeas ('CA46' and 'Top Crop') and lablab 'Rongai' and ii) determine the effect of fungicide seed treatment and *Bradyrhizobium* spp. inoculation on grain yield and above-ground biomass of the crops. The experimental design was a randomized complete block in a factorial of four pulse crops with and without rhizobium inoculation and a fungicide seed treatment. Although several research studies indicated that fungicide seed treatment and inoculation with *Bradyrhizobium* affected nodulation, nitrogen fixation and yield components, we found no significant effects of fungicide seed treatment and *Bradyrhizobium* inoculation on pulse grain yield.

The role of legumes worldwide includes food, nutrition and income generation among others. Malnutrition especially in children is common in many developing countries, leading to severe stunting and death in addition to high macro and micro nutrient deficiencies which legumes can

provide. Legumes such as cowpea can thrive in adverse environments like drought, making them a climate smart technology for hunger mitigation. Total protein intake per capita from pulses exceeds 10% in several countries, including Uganda (20%) and Ethiopia (15%), yet potential legume leaf utilization for protein and other nutrients has not been widely considered. This may be a rare source of nutritional and harvest versatility compared to common leafy vegetables. However, leaf removal effects on pulses and their grain yield are unclear. Additionally, little information is available on pulse leaf nutritional composition. We conducted a two-year experiment in central Iowa to determine 1) the effects of leaf removal rate on nutritive value of removed leaf tissue, and 2) the effects of leaf removal rate on subsequent grain yield. We found that mean concentration of nutrients in dry leaves was 229, 17832, 4461, 21991, 3702, 113, 205, 13, 86, 2806 mg kg^{-1} for crude protein, calcium, Mg, K, P, Mn, Fe, Cu, Zn and S. Unlike 2013, leaf removal percentage had significant impact on 2014 yield and the control had 20, 32, and 35% more yield than plots at 33, 66 and 99% leaf removal. Pulse leaves have excellent potential to supplement high-carbohydrate diets that are deficient in protein and micronutrients.

CHAPTER 1

LITERATURE REVIEW

Influence of *Bradyhizobium* Inoculation and Fungicide Treatment on Development and Yield of Selected Pulse Crops

Soybean

Soybean is the most economically important oilseed crop in the USA and is responsible for 90% of annual oilseed production (Ash, 2006). In American cultivars, *Bradyrhizobium* is the bacterial species specific for symbiotic nitrogen fixation with soybean. Unlike some legumes, soybean can derive most of its nitrogen requirements from symbiotic nitrogen fixation and its ability to produce high yields depends immensely on adequate biological nitrogen fixation (Duong *et al*., 1984). The processes of root nodulation and nitrogen fixation do not solely depend on biological factors (Halverson and Stacey, 1986). They also depend on a number of abiotic components such as soil profile and certain synthetic chemicals including biocides, fertilizers, insecticides and fungicides (Anderson, 1978; Gaur, 1980; Kundu and Trimohan, 1989).

Balasubramanian et al. (1987) noted that *Bradyrhizobia* introduction to the soil led to significant increase in nodulation and nodule dry weight. Singleton and Stockinger (1983) reported that 75% of effective nodules accounted for 95% of maximum N accumulation in soybeans. Similarly, Tien *et al*. (2002) and Lindermann and Glover (2003) also reported that up to 280 kg N ha^{-1} could be fixed symbiotically, accounting for about 70% of the total plant nitrogen requirement in soybeans. This ability to fix large sources of nitrogen depends on soybean's efficiency for symbiosis between plant roots and *Bradyrhizobium japonicum*

(Pueppke, 1992). Other factors that influence nodulation and nitrogen fixation following inoculation include;

History of soybean growth and previous inoculation

In fields where soybean was grown for the first time, dramatic increase in yields of up to 50% or more were observed (Duong *et al*., 1984 and Seneviratne *et al*., (2000). Schulz and Thelen (2008) found an average seed yield difference of 85.6 kg ha^{-1} between inoculated and non-inoculated plots from 14 site-years on which inoculant trials were performed on soils with soybean production history. Future inoculations in a field where *B. japonicum* was inoculated in previous years may not likely have as great an effect on soybean yield as initial or first time inoculation in that field. Schulz and Thelen (2008) explained that soil-borne populations of *B. japonicum* resided in such a field in the years that followed. The authors also hypothesized that there may not be significant differences in the effectiveness of particular commercial brands of inoculant except in plots where soybean has not been grown in the past. In an earlier study, Pasaribu *et al*. (1989) found only one site out of 11 to have yield response to inoculation even with compatible strains of *B. japonicum*. This clearly indicates the importance of using strains which are not only compatible but especially effective.

Inoculant form and strain

Soybean seed inoculant is usually available in granular, peat based and liquid forms. Often, liquid inoculations are known to have concentrations of up to 2 billion or more bacteria per gram which enables lower rates of application and more efficient use compared to other forms (Schulz and Thelen, 2008). Schulz and Thelen (2008) observed significant yield advantage

from using liquid inoculants compared to other forms of inoculant in fields with no previous soybean production. They also found that soils where soybean production was done for the first time had higher yield increases with liquid inoculant seed-application. Conversely, Bohner (2002) in Ontario, Canada and Beuerlein (2007) in Ohio on rotational soybean ground found liquid and peat-based carriers to have the same effects on yield. However, often times, a certain percentage of peat inoculant failed to properly adhere to the seeds or fell off during seed transportation or movement within the planter to the bottom of the hopper (Smith, 1992). It is worth mentioning that different strains of inoculant may have different effectiveness at nodulation. Cregan *et al*. (1989) reported that strains of *B. japonicum* from USDA 123 serogroup were very effective at nodulation yet much less effective at nitrogen fixation than those used in other soybean inoculations like USDA 110.

Successive inoculation

Successive inoculation increased yield thus making inoculation worthwhile to farmers and researchers (Beuerlein, 2005). On the other hand, Pederson et al. (2003) and Abendroth and Elmore (2006) hypothesized no yield increase from successive soybean inoculation in a number of trials. Schulz and Thelen (2008) also observed that yield significantly decreased by about 280 kg ha^{-1} (6%) at a soybean site with inoculation. Conley (2007) also noted decline in soybean yield with inoculation. Long periods without rain could partly be the reason for negative inoculant results especially in years where rainfall deficit occurred during pod fill stages (Schulz and Thelen, 2008). However, in the same study but at different sites, they also observed no significant differences in yield whether or not inoculant was applied.

Soil factors and native strains

In explanation of why inoculation may result in yield reduction or have no significant impact on yield, Graham (1992) and Abendroth and Elmore (2006) postulated that soil factors including water content, organic matter, pH and soil texture impacted the populations of *B. japonicum*. Daramola et al. (1994) further stressed that many soils usually harbored native Rhizobium strains of different competitiveness even before inoculation. Successive inoculation improved soybean root colonization of *B. japonicum* and helped overcome soil and environmental hindrances (Schulz and Thelen, 2008).

Long periods without rain caused the negative inoculant results on yield especially in the years where rainfall deficit occurred during pod filling stages (Schulz and Thelen, 2008). The authors further explained that soybean inoculant benefited grain yield most often when normal precipitation was prevalent during pod filling. Lawn and Brun (1974) also observed that nitrogen fixation by nodules peaked and then rapidly decreased when soybean pod filling begun. Thibodeau and Jaworski (1975) and Abendroth and Elmore (2006) also found nitrogen fixation to peak before early pod fill.

Schulz and Thelen (2008) hypothesized that nodule death and thus decline in nitrogen fixation may be because the plant-nodule relationship is a source-sink relationship. Therefore, as photosynthate is supplied to new seeds, it is diverted from nodule feeding especially under stressful conditions when resources are often limited. Plant depodding enabled longer maintenance of nodule activity whereas plant defoliation caused earlier decline in nitrogen fixation in nodules (Lawn and Brun, 1974). Schulz and Thelen (2008) further explained that inoculated plots with superior nodulation were likely to compete with developing fruit for leaf photosynthate especially during early pod filling. They also speculated that enhancement of early

season growth from inoculants in high yielding beans created large vegetative sink within the plant which led to low yield in cases of inadequate rain late in the growing season. However, indirect evidence of the existence of a source – sink hypothesis by Lawn and Brun (1974) and Schulze (2003) has not been supported with direct evidence to relate carbon assimilate competition by nodules and nitrogen fixation (Schulze *et al*., 2000).

Cowpea

In countries like Brazil, the cowpea agribusiness generates above 350 million US dollars per year (Freire *et al*., 1982). Effectively nodulated cowpea can derive up to 90% of its N requirements from nitrogen fixation to obtain maximum yield (Eaglesham *et al*., 1977). However, due to inadequate infectivity and efficacy of indigenous rhizobia in the soil, such potential may not always be realized (Singleton and Tavares, 1986). This calls for improvement of cowpea yields by using microbial inoculants (Fening and Danso, 2002). Cowpea can be nodulated by a large range of soil rhizobia (Martins *et al*., 2003). Several research studies on cowpea indicated that inoculation had significant impact on yield both positive and negative.

Increased yield with inoculation

There were increased cowpea yields with inoculation (Danso and Owiredu, 1988). Figueiredo *et al*. (1999) also demonstrated the importance of inoculation in stressed cowpeas. Martins *et al.* (2003) hypothesized that cowpea inoculation in dry and semi-arid regions was a feasible practice. He further explained that soils in such areas had low rhizobium populations at sowing time and thus use of inoculation significantly increased grain yield. Inoculation has also proved to be of great importance in growing conditions with existing environmental stress.

Figueiredo *et al.* (1999) noted that at negative water potential, inoculated plants (strain EI-6 was used in the study) had higher nitrogen content, nodule size, shoot/root ratio, concentration of ureide-N and leghaemoglobin (LHb) compared to the uninoculated plants. Walker and Miller (1986) explained that negative water potential affected nitrogen fixation by plants and resulted in reduced N_2ase activity, weight and number of nodules.

Research also shows reduced yield may at times ensue inoculation. Figueiredo *et al*. (1999) stressed that energy requirements for N_2 fixation in cowpea following inoculation demanded dry matter reduction of about 15%. Further, the nitrogen fixation process demands continuous supply of carbohydrates (Neves, 1981), in such scenarios, inoculation may negatively affect dry matter and yield. According to several studies, a number of factors may influence the outcomes of inoculation.

Indigenous rhizobia in the soil

Fening and Danso (2002) hypothesized that existence, abundance and effectiveness of indigenous rhizobia in the soil was of great importance in order to ascertain the potential for improving biological nitrogen fixation through inoculation. They explained that fields had great variation in the numerical size of rhizobia populations. The presence of an appropriate host whether continuing or periodic was a major determinant of whether inoculation was valuable for nodulation and nitrogen fixation (Rupela *et al*., 1987; Woomer *et al*., 1988). The fact that cowpea can be inoculated by a wide range of soil rhizobia made it difficult to establish an effective inoculant strain in field conditions (Martins *et al*., 2003). Therefore, the competitive superiority of an introduced strain influenced its transient success at forming nodules given that naturally existing rhizobia in the soil are more persistent than introduced strains (Fening and

Danso, 2002). To complement Fening and Danso (2002), earlier studied by Strijdom *et al*. (1988) and Strijdom (1998) found *Bradyrhizobia* inoculation of cowpea to be of little benefit in soils that had already existing indigenous rhizobia. The presence of effective indigenous inoculant species populations in the soil caused lack of cowpea response to inoculation (Bushby, 1984; Danso and Owiredu, 1988; Fening and Danso, 2001). Additionally, exotic strains had high mortality following their introduction in the soil which may have led to unsuccessful nodulation (Brockwell *et al*., 1987). To solve this challenge, Danso and Owiedu (1988) hypothesized that inoculating with high numbers of *Bradyrhizobia* gave positive results.

Rhizobia 'promiscuity'

Singleton *et al*. (1992) described rhizobia as 'promiscuous' and with the capability of nodulating a wide range of legumes, though, with poor effectiveness. Such characteristics may negatively impact the establishment of other more efficient inoculant strains (Mpepereki *et al*., 1996). This is because the already existing rhizobia in the soil act as a barrier to not only nodule formation optimization but also to biological nitrogen fixation (Mpepereki *et al*., 1996). It is therefore important to study and understand ecological parameters such as competitiveness and soil survival of isolates as well as dynamics of rhizobial populations before inoculation (Martins *et al*., 1997). In context of Rhizobium spp., competitiveness refers to the ability of a strain to form nodules through successful competition with the various strains already present in the seed or root environment (Simon *et al.,* 1997).

Soil properties

Rhizobial populations may be influenced by soil fertility, physical properties such as pH and soil clay content, biotic factors like prevalence of predators and distribution of the host plant and other climatic factors like rainfall and temperature (Hirsch, 1996) . Awonaike *et al*. (1990) reported no improvement in cowpea nodulation following inoculation in tropical soils. Further, Toro (1996) hypothesized that nodulation depended on adequate amounts of phosphorous and calcium but was adversely affected by manganese and aluminum NO_{-3}. Xavier *et al*. (1998) demonstrated a strong correlation between aluminum concentration and the competitiveness of rhizobium strains in soil. Similar to Hirsch (1996), Law *et al*. (2006) also hypothesized that hot and dry environmental conditions caused poor survival of some *Bradyrhizobia* strains. He found that sandy to sandy-loam soil with low pH was more favorable (for strain CB756 used in that study) than loamy sand or sandy clay loam soil with higher pH. In addition, a previous study by Bushby (1984) in Australia reported that strain CB756 failed to grow in heavy clay soils and was poorly competitive against indigenous populations.

Management practices

Several studies document the value of management practices towards nodulation and nitrogen fixation. Management practices that encouraged effective nodulation by indigenous populations or the enrichment of effective and competitive strains were more valuable than further inoculation (Thies *et al*., 1995; Van Kessel and Hartley, 2000). Fening and Danso (2002) reported that agricultural practices contributed to the field dominance of indigenous strains. They also suggested the potential to best increase nitrogen fixation through exploitation of characteristics of rhizobial populations already existing in the soil rather than new inoculation.

Law *et al.* (2006) emphasized the importance of determining symbiotic and structural characteristics of soil rhizobial populations and how suitable they would be for an introduced crop. However, Denison and Kiers (2004) noted that host plants exerted physiological sanctions on ineffective nodules but favored effective nodules and their rhizobia. This makes it beneficial to transform originally ineffective populations on one legume like peanut to populations effective on two legumes grown in the same area for instance, both peanuts and cowpeas (Denison and Kiers, 2004).

Energy requirements for nitrogen fixation

Unlike Fening and Danso (2002), Kang *et al*. (1977), Rhodes and Nangju (1979) and Awonaike *et al*. (1990) attributed the lack of cowpea response to inoculation to reasons other than predominance of highly effective strains in the soil. Figueiredo *et al*. (1999) explained that energy requirements for N_2 fixation in cowpea demanded dry matter reduction of about 15%. In addition, the nitrogen fixation process demanded continuous supply of carbohydrates which could lead to reduction in yield following inoculation (Neves, 1981).

Climate

One of the greatest pressures in plant evolution is water shortage (Boyer, 1980). The plants' ability to survive with water stress is a major determining factor in the distribution and productivity of cultivated plants (Boyer, 1980). In numerous crop species, nitrogen fixation has been shown to be sensitive to reductions in water availability in the soil (Sinclair *et al*., 1987; Guerin *et al*., 1991; Gonzalez *et al*., 1995). Venkateswarlu *et al*. (1989) and Stamford *et al*. (1990) demonstrated that N accumulation in cowpea was affected by water stress. Regardless of

cowpea's tolerance to prolonged drought, the crop was still quite susceptible to lack of water/moisture especially during the flowering phase (Venkateswarlu *et al*., 1989 and Stamford *et al*., 1990). Drought had the selection pressure to either stimulate adaptive reactions that aid survival in times of water deficit or weaken a plant's vital functions (Pimentel *et al*., 1990). Drought is also one of the environmental stresses which can cause considerable reductions in N_2 fixation (Sinclair *et al*., 1987). It was however not clear which particular function of a stressed plant affected the nodules (Streeter, 1993). It is therefore important to select cowpea rhizobial strains according to their potential to recover and withstand water stress (Walker and Miller Jr, 1986 and Stamford *et al*., 1990).

It is worth noting that both water excess and deficiency can directly hinder the development of cowpea and affect biological nitrogen fixation by affecting the physiological activities of microorganisms and their survival (Figueiredo et al., 1998). Nitrogen fixation was inhibited by excess water in the soil because of inability of rhizobia cells to survive biological and physical-chemical alterations that arise from water variations (Alexander and Osa-Afiana, 1982). Although the effect of rhizosphere is not clear, Athar and Johnson (1990) hypothesized that the rhizosphere contributed to the selection of competitive but ineffective rhizobial strains that yielded no effect on nodulation or nitrogen fixation in cowpeas.

Lablab

The ability of inoculation to bring about nodulation in lablab may be influenced by a number of factors. Although nodulation and nitrogen fixation are expected following inoculation, inoculation may not always enhance nodulation (Rotimi, 1972). In his experiments, he observed no simulation on nodulation from inoculation. Dunigan *et al*. (1984), Howle *et al*. (1987) and

Chemining'wa *et al*. (2004) also documented the lack of yield improvement following inoculation. Laws and Graves (2005) hypothesized that inoculation negatively affected nodulation. When sufficient levels of nitrogen were already present in the soil, nodulation was inhibited (Gentili and Huss-Dabell, 2002 and 2003; Laws and Graves, 2005). In other research studies, added inoculant may have had inhibitory effects to nodulation as well as nitrogen fixation (Floor, 1985; Chemining'wa *et al*., 2004; Taylor *et al*., 2005). On the other hand, Otieno *et al*. (2007) postulated that rhizobia inoculation significantly increased the number of nodules per plant, increased nodule dry weight but had no effect on shoot dry matter. The effects of inoculation in lablab are dependent on a number of factors.

Manure Vs Nitrogen fertilizer

In seasons with torrential rains, the number of root nodules per plant in lablab were significantly reduced by the application of nitrogen fertilizer though manure application had no significant effect (Otieno *et al*., 2007). Unlike nitrogen fertilizer, manure application did not improve or decrease nodulation in lablab (Otieno *et al*., 2007). This may be explained by slow mineralization of manure hence slow nitrogen release. Otieno *et al*. (2007) further reported that an average across a number of legume species showed higher nodule numbers from plants which were treated with manure relative to the control. The presence of phosphorous in manure probably resulted in the positive effect of manure on nodulation compared to the control (Otieno *et al*., 2007). Similarly, Toro (1996) hypothesized that nodulation depended on adequate amounts of phosphorous and calcium. Floor (1985) also reported improvement in nodulation with presence of phosphorous.

Native rhizobia and cultivar strain interaction

Otieno *et al*. (2007) observed that effectiveness of rhizobia strains to form nodules was immensely influenced by whether the rhizobia strains were either absent in the soil or not suited to the legume species. Caldwell (1966) referred to the latter as poor cultivar and strain interaction. Several researchers documented a number of other factors that affected nodulation following inoculation and these include the presence of native and effective rhizobia strains in the soil (Ham *et al*., 1971); high soil nitrogen (Sparrow and Ham, 1983) and other climatic factors such as drought which affected host and rhizobia symbiosis and influenced the survival of rhizobia and nodulation process (Graham, 1992).

Nutritional composition of pulse legume leaves and the impact of leaf removal on yield

Cowpea

Cowpea [*Vigna unguiculata* (L.) Walp.] is a stress tolerant legume, vegetable and fodder crop commonly grown in warm or hot regions. Africa, Asia and a number of countries in Central and South America (especially semi-arid northeastern Brazil) lead in cowpea production. The crop is widely adapted and typically grown in hot low elevation equatorial and subtropical regions (Ehlers and Hall, 1997). However, the crop can give satisfactory yield under greater diversity of soils, climate and cultures compared to other legumes (Ligon, 1958).

According to Ng and Padulosi (1988), West Africa is the primary center of diversity for the domesticated cowpea while India is a secondary center. Given its considerable tolerance to drought and high temperatures, cowpeas often produced higher yields compared to other pulse species. Up to 1000 kg ha^{-1} of dry grain can be obtained with 181 mm of rainfall despite high

evaporation in Sahel regions (Hall, 2004). Additionally, cowpea is tolerant of low fertility soils because of its high nitrogen fixation rates (Elowad and Hall, 1987), its ability to withstand both high and low soil pH (Fery, 1990), and the ability to form mycorrhizal associations for improved water and P uptake (Kwapata and Hall,1990).

Dry grain production often is the primary goal from cowpea plantings, however leaves (in many East African areas), fresh pods (in Asian and Caribbean areas) and fresh peas (Senegal and southeastern US) are also commonly used for human consumption (Ehlers and Hall, 1997). The crop is also used for green manure and fodder for ruminant livestock (Ehlers and Hall, 1997).

Depending on region and importance of the crop, cowpea is sole-cropped or intercropped within rows of other plants. In Africa, it is intercropped with sorghum [*Sorghum bicolor* (L.) Moench], pearl millet [*Pennisetum glaucum* (L.) R. Br.], maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz) or cotton (*Gossypium barbadense* L.) (Blade *et al*., 1997). Increasing consumer demand has contributed to greater commercial sole cropping of cowpeas, especially to meet the needs of growing urban populations (Ehlers and Hall, 1997).

In addition to fixing nitrogen to the soil and thus increasing fertility, numerous studies report that cowpea helps to control striga. *Striga hermonthica* Benth. (Orobanchaceae) commonly known as striga, is a parasitic weed whose infestation in cereal fields can cause yield losses of up to 100% depending on the susceptibility of the specific host genotype (Berner *et al*., 1995). Striga infestation is known to be more severe in agricultural systems with poor soil fertility and low fertilizer input (Debra *et al*., 1998). Intercropping cowpeas with other crops can reduce seasonal emergence of striga for instance, by up to 15% in maize. Maize intercropped

with cowpea grows taller with heights enhanced up to 60% and total grain yield improvements ranging from 40% to about 50% (Midega et al., 2014).

The nutritional composition of cowpea seed is similar to that of common bean (*Phaseolus vulgaris* L.) (Bressani, 1985). However, cowpeas typically have higher folic acid and lower antinutritional and flatulence producing factors than beans (Ehlers and Hall, 1997). Plant breeders have been quite successful in improving cowpea varieties for early maturing cultivars, regionally preferred seed types, drought tolerance and disease and insect resistance (Ehlers and Hall, 1997). These improved varieties have enabled regions like East Africa with long and short rain seasons to grow early maturing cowpea cultivars in seasons that previously were considered too short for most of other crops to be grown (Ehlers and Hall, 1997). Early maturing cultivars also have the ability to escape insect pest damage by pod bugs and flower thrips. Cardinali *et al*. (1995) observed that some cowpea varieties had high resistance to aphids, which may be related to high flavonoid levels.

A study by Halliwell (1994) postulated that the existence of free oxygen radicals and lipid peroxidation may be involved in several pathological conditions including cancer and other chronic inflammations yet Kandaswami and Middleton (1994) hypothesized that flavonoids may protect body tissues against free oxygen radicals and lipid peroxidation because of antioxidant properties. Cowpea is high in such flavonoids. It is important for grain cowpea to suit the preferences of the local or international market to which sale is intended. Businesses producing cowpea flour prefer the large white-seeded varieties (McWatters, 1985); blackeyed peas (large white grain with black pigment/eye around the hilum) on the other hand are another valuable class that is traded internationally (Ehlers and Hall, 1997).

Phenology characteristics

Cowpea plant architecture varies by location and planting date, especially when there is sensitivity to photoperiod (Ehlers and Hall, 1997). This explains why cultivars that are known for early maturity and erect growth habits in short-day environments may show significant delays in flowering when grown in long-day environments and may also be prostrate (spreading) in growth habit. Cowpeas are a short day plant and many cowpea accessions are photoperiod sensitive (Ehlers and Hall, 1997; Craufurd *et al*., 1997). Temperature is known to modify the degree of sensitivity to photoperiod for some genotypes (Wein and Summerfield, 1980; Ehlers and Hall, 1996). The influence of temperature on photoperiod sensitivity is particularly acute when pod ripening coincides with the end of the rainy season (Steele and Mehra, 1980). This not only enables pods to survive damage from excessive moisture but also from pathogens (Ehlers and Hall, 1997).

Early flowering enables cowpea cultivars to escape drought in some regions and thus produce a more stable grain yield (Hall *et al*., 2000). However, these early flowering cultivars are known for their sensitivity to drought occurrence during early stages of reproductive development (Cisse *et al*., 1995). Conversely, Hall *et al*. (2000) noted that delayed senescence can enhance drought adaptation of early cowpea cultivars by development of a potentially high yielding second pod flush when drought decreased the first pod set.

In the US, cowpea production is not new and prior to the Second World War, the crop was a major forage for horses and cattle, hence the common name cowpea. Today, cowpea continues to be grown for grain production and as a soil-building cover crop (Ehlers *et al*., 1998). Cover crops improve soil properties including organic matter accumulation and thus fertility, temperature, tilth and water holding capacity (Abdul-Baki *et al*., 1997; Teasdale and Shirley,

1998). Cover crops may also help in pest control by suppressing weeds (Harrison et al., 2006). They explained cowpea traits such as heat and drought tolerance, ability to grow well in sandy, acidic and poor soils which make the crop an excellent warm season cover crop.

Cowpea defoliation

Utilization of cowpea as a leafy vegetable as well as for grain may be a source of nutritional and harvest versatility that is rare with most other commonly grown vegetable crops such as cabbage or lettuce (Buhenheim and Mitchell, 1987, 1988). With proper management, cowpeas leaves can be harvested without adversely impacting seed yield (Imungi and Potter, 1987; Akubdabweni *et al*., 1989). However, even when harvest of leaves is limited, a negative effect on harvested seed yield at maturity can still occur (Bubenheim et al., 1990 and Nielsen *et al*., 1994). The effect of leaf harvesting on yield decline may be attributed to factors such as differences in cultivars. Bittenbender *et al*. (1984) noted that seed yield of some cowpea cultivars is adversely affected by leaf harvest whereas other cultivars may not be affected at all. Wein and Tayo (1978) further noted that after defoliation, seed yield of leafy and determinate types suffers more reduction than that of indeterminate types.

Seed and Pod yield

Bubenheim *et al*. (1990) reported that harvest strategy does not impact biomass accumulation or diversion of the plant's vegetative portion. In trials were both leaves and seeds were harvested and other trials where only seeds were harvested, the seeds were obtained from both trials after 75 days from germination. Bubenheim and Nielsen (1990) also found that seed yield, seed and pod number per plant were severely affected by partial defoliation because the

reduction in source leaves limited the reproductive sink size (seed number per plant). Size of individual seeds was not affected and the mixed-harvest strategy of both leaves and seeds only increased by 2 more days in reaching the time to harvest. According to Barret (1987) removal of too many young leaves at once impaired seed yield whereas removal of the oldest leaves increased it. Timing of leaf removal also greatly affected the plant's ability to recover from defoliation (Barret, 1987). Studies by Karikari and Molatakgosi (1999) showed that cowpea grain yield was dependent on both cultivar and leaf harvesting intensity and in their study, up to 50% leaf harvesting of leaves increased grain yield of 'Blackeye' and 'Twana' but removing 75% of leaves decreased grain yields.

Saidi *et al.* (2007) explained that delaying initiation of leaf harvest to the fifth week after emergence reduced grain losses. Grain yield was lowest in plants were leaf harvest was initiated in the second week after emergence or under more frequent 7-day leaf harvesting intervals (Saidi *et al*., 2007). These authors further explained that delaying the first leaf harvest or reducing the harvesting frequency left more leaves on plants, allowing a larger photosynthetic surface. With a slow rate of leaf formation given that young tender leaves were harvested, this could have led to better accumulation of C and N reserves for subsequent grain production. Saidi *et al*. (2007) reported that higher leaf yield led to decreased grain yield. This varied from other studies that showed that leaf removal had detrimental effects on subsequent crop yields (Nielsen *et al*., 1994; Muir *et al*., 2005). Saidi *et al*. (2007) postulated that low grain yield following high vegetative leaf yields may be due to changes in photosynthesis and source –sink relationships within the plant. Hoogesteger and Karlsson (1992) hypothesized that defoliation altered photosynthesis directly through changing source-sink relations, thus when leaves were harvested, photosynthates

were directed towards development of new leaves at the expense of being used in grain production.

Enyi (1975) reported that defoliation reduced the dry weight of stems, pods, grains and size of individual grains. Enyi (1975a) further explained that the adverse effects of defoliation were more pronounced with complete defoliation than when only half of the number of leaves was harvested from the plant. The greatest reduction in grain yield was observed when plants were defoliated during early podding stage (Enyi, 1975). According to Enyi (1975b), during early leaf stages, assimilates produced by the leaves are mainly used for development of main stems and new leaves since leaf harvesting at this stage reduces weight of side and main stems, number of side branches and plant height. Enyi (1975b) further explained that during reproductive stages, assimilates produced by leaves are used for pod initiation and growth, thus harvesting of leaves at this stage is detrimental to pod numbers and filling of pods due to an inadequate supply of assimilates.

Removal of cowpea leaves by various methods and different percentages caused significant reductions in seed yields (Barret, 1987). Reduction of leaf yields by 80% at flowering decreased grain yield to 946 kg ha^{-1}, 62.3% of the control because plants were unable to compensate for this severe reduction in leaf area (Demooy and Demooy, 1989). The authors further explained that defoliation affected seed size and number of pods per plant but not the number of seeds per pod for ER-7 cowpea cultivar. While many studies indicate defoliation to be detrimental, Stratus (1988) found that defoliation increased seed yield.

Biomass partitioning

Removal of young expanding trifoliate leaves during the vegetative stage just prior to flowering suppressed total plant biomass and altered partitioning compared to plants grown

conventionally, only for seed harvest without leaf removal (Bubenheim *et al*., 1990). Their study showed that periodic partial defoliation stimulated leaf production with 68% of cumulative biomass in form of leaves compared to 57% for conventional vegetative stage plants. Plants from which leaves were harvested during growth had twice as much overall leaf dry weight compared to plants grown solely for grain harvest (Mitchell *et al*., 1990). Leaf harvesting at 5 or 7weeks after planting did not affect leaf or stem weight per plant at maturity but had detrimental effects on grain yield (Nielsen *et al*., 1994) According to Saidi *et al*. (2007), total biomass and biomass partitioning to roots, stems, pods and leaves was affected by leaf harvesting initiation and frequency. Defoliation accelerated shoot growth at the expense of root growth which might increase susceptibility to drought stress (Wilson, 1988).

Frequency and timing of leaf harvesting

The timing of leaf removal greatly impacts cowpea plants ability to recover from leaf harvesting (Barret, 1987). Both time from crop emergence to first leaf harvest and leaf harvesting frequency had an impact on fresh vegetable leaf yield of Ex-Luanda and Kathoka cowpea cultivars (Saidi *et al*., 2007).Weekly leaf harvesting gave higher leaf yield but lower grain compared to harvesting biweekly (Saidi *et al*., 2007). Vegetative stage defoliation of cowpeas up to 50% did not influence grain yield (Wein and Tayo, 1978). Saidi *et al*. (2007) further explained that delayed initiation of leaf harvesting reduced the time period between leaf harvest initiation and flowering, resulting in the reduction in number of harvests that could be made. Because defoliation stimulates leaf production in cowpeas (Bubenheim *et al*., 1990), high frequency (weekly) leaf harvesting yielded more leaves than biweekly harvesting (Saidi *et al*., 2007).

Nutritional composition

Carbohydrate content was greater in seeds compared to leaves (Bubenheim *et al*., 1990). However, the carbohydrate content in leaves increased with leaf age thus leaves harvested at a later stage had greater carbohydrate content than those harvested earlier. Conversely, protein content in older leaves was not different from that in grains or seeds but average fat content was greater in leaf tissue than seed and, age did not affect the latter. Ohler *et al*. (1996) reported that total dietary fiber content of leaves increased from 19% to 26% when the time to harvest was increased from 20 to 50 days. Dietary fiber, also known as roughage includes all parts of a plant food that the body cannot digest or even absorb (Hans *et al*., 1990). Dietary fiber is important in lowering cholesterol levels, reducing heart health problems, blood pressure and inflammation. It also plays an important role in normalizing bowl movements and preventing colon diseases.

Bryant *et al*. (1983) and Tuomi *et al*. (1984, 1990) postulated that a carbon-nutrient balance within plants was responsible for induction of chemical defenses within the plant. Hikosaka *et al*. (2005) explained that new leaves that regenerated after harvesting had lower nitrogen concentration but greater amounts of secondary compounds. Secondary compounds such as phenolics are produced from excessive carbon because harvested leaves decrease the nitrogen concentration of the whole plant. Hikosaka *et al*. (2005) further illustrated that defoliation did not cause nitrogen deficiency in the long term because of increased rates of nitrogen uptake. On the contrary, Tuomi *et al*. (1988), Kamata *et al*. (1996) and Kudo (1996) explained that leaf nitrogen content in woody species decreased or did not change after defoliation. Hikosaka *et al*. (2005), Nowak and Caldwell (1984), and Anten and Ackerly (2001)

hypothesized that an increase in leaf N concentration content would enhance leaf photosynthesis (compensatory synthesis).

High soil temperatures and greater day/night variations in soil temperatures resulted in lower seed yields, fewer peduncles and reduced vegetative growth (Warrag and Hall, 1984). Cowpea grain yield is greatly influenced by environmental factors, including climate, and soil temperature. Other factors impacting cowpea grain yield following plant defoliation include leaf harvesting intensity (Enyi, 1975a; Karikari and Molatakgosi, 1999), timing of defoliation and defoliation frequency (Barret, 1987; Saidi *et al*., 2007).

Lablab

Lablab purpureus (L.) Sweet, previously named *Dolichos lablab*, is a widespread food crop in Africa and Asia with edible leaves (young and tender), pods and seeds. Since 1962, the cultivar Rongai has been widely grown in Australia and is currently one of the major leguminous forage and green manure crops in that part of the world (Cameron, 1988).

Lablab is a frost-sensitive summer annual or short lived perennial. The herbaceous plant vigorously trails and twines and is resistant to disease and insect pests (Milford and Minson, 1967; Cameron, 1988). Stems trail upwards and can reach up to 3m and are robust. Lablab is known for being one of the most agro-morphologically diverse (Pengelly and Maass, 2001; Mohan and Aghora, 2006; Islam, 2008) and versatile tropical legume species as a pulse, vegetable (green bean, pod, leaf), forage, green manure, herbal medicine and ornamental (Adebisi and Bosch, 2004; NRC, 2006) and also recently for its bio-functional use in pharmaceutical or nutraceutical (Maass *et al*., 2010).

The crop is used for forage, especially the older leaves. Hay cut at a young and leafy stage is nutritionally comparable to alfalfa although it is considerably less digestible (Baloyi and Ayodele, 2013). Lablab is a prolific food crop with the ability to thrive on relatively low fertility soils. The crop is also known for high yields and drought resistance unlike many other legumes. Murphy and Colucci (1999) mentioned that due to lablab's high biomass forage yields in drought, it is now commonly preferred to cowpeas. The legume fixes nitrogen through symbiosis with bacteria in root nodules and is easy for farmers to establish and manage as a cover crop. This cover helps to reduce soil erosion, conserve the soil, improve organic matter content as well as compete with weeds (Humphreys,1994 and Schaaffhausen, 1963a, b).

The crop grows well with intercropping or sole-planting and serves as a great cover crop. Lablab is known to have high nutritive value with 20 to 28% crude protein, 6-7% lysine, good quality protein and seeds with high energy composition (Baloyi and Ayodele, 2013). Compared to other legumes, lablab is also one of the best sources of iron (155mg/100g of leaves dry weight) according to (Deka and Sarkar, 1990; Norton and Poppi, 1995). Agishi (1991) noted that lablab was a good feed for monogastric animals since its leaves do not contain tannins. Lablab was shown to increase weight of Zebu cattle (Nelore Breed) during the dry season and milk production (when added to maize sillage) in dairy cattle in experiments done in Sao Paulo, Brazil (Murphy and Colucci, 1990).

Cultivar

Lablab varieties may differ in morphological characteristics including pod shape, size and color as well as their seasonal adaptation (Maass *et al*., 2010). The Rongai cultivar was derived from material collected from Rongai district of Kenya (Cameron, 1988) and was then released in Australia in 1962 (Wilson and Murtagh, 1962). It is an annual or short lived perennial and stems

trail and reach 3 to 6 m in length. Flowering depends on photoperiod, not temperature (Schaaffhausen, 1962). Leaves are trifoliate and broad ovate-rhomboid. (Murtagh and Dougherty, 1968; Cameron, 1988). Rongai is a late maturing cultivar with white flowers that continue to grow until damaged by frost or harvest (Maass *et al*., 2003). Whitbread and Pengelly (2004) demonstrated that long vegetative phases make Rongai prone to early frosts or drought and delayed production.

Lablab is adaptable to drought and has the ability to grow in a wide range of environments. Even in dry seasons, lablab had the potential to stay green and can provide up to 6 Mt DM ha^{-1} (Murphy and Colucci, 1999). For a crop whose leaves can be consumed as vegetables and forage, there is concern about its response to defoliation. Salisbury and Ross (1992) and Saidi *et al*. (2010, explained that response of plants to defoliation depended on a number of factors including intensity of defoliation, frequency and the timing of forage removal. In addition, Rahman *et al*. (2008) noted that leaf harvesting procedures and handling had the potential to reduce yield of essential crop components.

Grain yield

Ogedegbe et al. (2012) noted that cutting or grazing lablab did not necessarily prevent seed production as most lablab producers seemed to believe. However, leaf harvesting or grazing between 12 to 18 weeks or at 18 weeks after planting led to lower seed yield compared to leaf harvesting at maturity, implying that lablab leaves should be cut later in the season at or after 18 weeks if seed production is desired (Ogedegbe et al., 2012). Ogedegbe et al. (2012) stated that the ability to produce seed could be attributed to a number of other factors including moisture stress that can hinder regrowth and other important physiological processes. In support,

El-Shatnawi *et al*. (2003) attributed lack of seed production in late cut wall barley (*Hordeum murinum* L. ssp. *murinum)* to rapid soil moisture reduction which decreased water status in plant tissues and eventually had negative impact on cell division and enlargement. In a research study by Wood (1983), he found that defoliation generally decreased grain yield and seed weight in lablab.

Biomass/ nutrient composition

The stem has greater NDF than the leaf due to greater lignin and fiber concentration (Karachi, 1997). The ADF is a good predictor of *in vitro* digestibility in many tropical legumes (Shehu *et al*., 2001). Defoliation of lablab by removing 52% and 76% of above ground biomass increased lignin and lowered N concentration (Nyambati, 2001). He reported defoliation of mucuna and lablab reduced total plant biomass and that biomass for undefoliated lablab plants was three times more than that of defoliated plants. Other research documented that leaves harvested at an early stage had little or no effect on crude protein and mineral contents in leaves at later stages of growth like flowering (Baloyi and Ayodele, 2013).

Crude protein concentration was higher in leaves harvested at early stages of growth than those harvested later in the season, indicating the importance of early harvest for improved nutritive value (Baloyi and Ayodele, 2013). Miller-Cebert *el al*. (2009) reported similar results for canola leaves that indicated higher protein content at pre-bolting stage than rosette and blooming stages. Defoliation reduced lablab leaf and stem N, Ca, and P concentration but increased lignin concentration (Nyambati, 2002). The study also showed that defoliation had no effect on K concentration. Mineral content of leaves was higher in leaves picked at an earlier

stage compared to those at a later stage of development (Baloyi and Ayodele, 2013). Deka and Sarkar (1990) concluded that the nutrient composition of legumes declined as the plant matured.

Soybean

Soybean [*Glycine max* (L.) Merr.] is a legume native to Asia. Soybean production has spread throughout the world and the crop is largely grown as a pulse for food, feed, and oil production with some accrual benefits of biodiesel. The crop has trifoliate leaves with stems, pods and leaves that have fine brown or gray simple trichomes. The plant has white, pink or purple flowers and seed coat color also ranges from black, brown, yellow, green and many others.

Soybean may be intercropped or sole cropped, which is the norm in mechanized agriculture. It thrives well in regions with hot summers and can grow well in a wide range of soils. However, optimum growth is obtained in moist alluvial soils with good organic content. Detrimental effects on yield, biomass, chemical and nutritional composition caused by defoliation are more prominent during reproductive plant phases rather than vegetative stages (Fehr *et al*., 1983; Hintz *et al*, 1991). However, defoliation at vegetative stage can also have impact the plant.

Seed yield

Seed number, size, protein, carbohydrate and oil broadly comprise soybean yield (Proulx and Naeve, 2009). Fehr *et al*. (1983) observed that defoliation during reproductive stage led to yield reduction unlike defoliation at vegetative stage that was found to have no effect on seed yield. Hintz *et al*. (1991), assumed that defoliation at vegetative stage (V3 and V6)

regardless of intensity had no impact on soybean seed yield. Hintz and Fehr (1990) also demonstrated that plants completely defoliated during vegetative development did not have reduction in seed yield unless the plants were injured by cuttings made on their stems. Burton *et al*. (1995) and Fujita *et al*. (1988) showed that defoliation decreased nitrogen fixation and thus the available N for seed growth. Mcalister and Krober (1958) demonstrated that reducing leaves by 80% was responsible for pod and seed abortion and that 40 and 80% leaf removal significantly lowered seed yield. Seeds also had lighter weight due to a reduced leaf area to supple photosynthate (McAlister and Krober, 1958). Todd and Morgan (1972) and Grymes *et al*.(1999) observed that soybean yield decreased as percentage defoliation increased. Fehr et al. (1977) also reported greater yield loss with defoliation at R5 than at R2. Grymes *et al*. (1999) attributed yield loss to reduction in seed weight caused by defoliation. A linear reduction in seed weight was observed as defoliation level increased. Lovvorn and Smith (1943) study on soybean defoliation reported that complete defoliation at any frequency was detrimental to growth and productivity.

Lovvorn and Smith (1943) explained that medium defoliation had significantly higher yields than either light or severe defoliation. Johnston and Pendleton (1968) observed that leaf removal from a certain area of the plant generally decreased seed yields within that area and other areas of the plant. Their study further explained that removal of top leaves caused 17% reduction in seed yield compared to 4% and 22% yield reductions caused by bottom and middle leaf removal respectively. However numerous studies demonstrated that both high level of defoliation at ‘vegetative’ stage or low defoliation at ‘reproductive’ stage sometimes decreased yield (McAlister and Krober, 1958; Todd and Morgan, 1972; Pickle and Caviness, 1983). Defoliation of 67% and 100% at vegetative stages (V1- V8) did not reduce yield (Begun and

Eden, 1965; Gazzoni, 1974; Gazzoni and Minor, 1979). However, continuous reduction in yield was noted with defoliation at reproductive stages (R1-R8), (Todd and Morgan, 1972). Conversely, Hammond (1988) demonstrated that soybean unifoliate defoliation at vegetative stage V_1 had no detrimental effect on yield but actually slightly increased yield. (Hammond, 1988) further explained in his study that number of seeds increased with 0 to 43.1% defoliation until 86.1% defoliation and that 50% defoliation had no significant impact on yield. Higley (1992), higher yields at earlier stages of soybean defoliation were the result of compensation for reduced photosynthetic leaf area by delaying senescence and delaying the decline of photosynthetic rates associated with aging thus sustaining photosynthate production later in the growing season. Similarly, low to medium crop defoliation at vegetative stage did not affect seed yield (Gould, 1960; Turnipseed, 1972; Gazzoni and Minor, 1979).

Biomass

Defoliation significantly affected plant leaf area and dry weights at R4 stage (Browde *et al*., 1994). They reported that leaf removal caused linear reductions in leaf area and in leaf dry weight for 2 consecutive years. They also demonstrated that defoliation caused greater decreases in canopy quantity and quality when soybeans were injured by herbicide. In earlier studies, both determinate and semideterminate soybean strain yields reduced with defoliation (Pickle and Caviness, 1983). Defoliation affected soybean dry matter accumulation by primarily reducing the effective leaf area for light interception and carbon fixation. Higley (1992) and Klubertanz (1996) reported that upper canopy defoliation resulted in increased apparent photosynthesis of the remaining lower leaves leading to delayed senescence of lower leaves due to increased sunlight exposure. Further, Gazzoni and Moscardi (1997) noted that soybeans had intense

compensatory leaf growth when low(33%) to medium (67%) defoliations were done at vegetative stages while defoliation at R6 reduced the intensity of natural leaf area loss.

Nutrient composition

A number of research studies document the effect of soybean defoliation. Defoliation greatly reduced oil concentration (Proulx and Naeve, 2009). Mcalister and Krober (1958) however noted that the iodine number of oil in seeds was higher in plants whose food supply was limited by defoliation. Defoliation resulted in seeds with lower protein concentration but higher oil concentration (Proulx and Naeve, 2009). Mcalister and Krober (1958) reported that oil and protein content were lowered by 80% plant defoliation. Burton *et al*. (1995) and Lawn and Brun (1974) reported unchanged seed protein content with defoliation. Conversely, Burton *et al*. (1995) reported decreased seed protein and oil content following defoliation. Burton *et al*. (1995) explained that defoliation reduced vegetative N remobilization which could have caused low protein concentrations. Mcalister and Krober (1958) reported that oil and protein content were lowered by 80% plant defoliation. Burton *et al*. (1994) reported that defoliation had no significant effect on seed protein content but reduced N concentrations in the seeds. Lawn and Brun (1974) further explained that reduction in seed protein content was because partial defoliation removed stored N and also reduced nitrogen fixation as a result of decreased photosynthate to the root nodules. On the contrary, Eckel *et al*. (1992) found increases in seed N concentration when soybeans were defoliated by insects. However, in that study, pods were insect damaged and the increased N may have been due to reduction in plant sink size. Bayne *et al* (1984) demonstrated that N and P concentrations in the leaves increased with severe defoliation.

Stage of defoliation and frequency

Turnipseed (1972) reported that seed weight was linked to stage of defoliation. He also observed more seed weight reduction in soybean plants defoliated at pod fill but not those defoliated at bloom stage. Board and Harville (1993) and Goli and Weaver (1986) also reported that defoliation at early reproductive stages up to R was more related to reduced pod numbers while defoliation at R5 and R6 was related to reduced seed weight. Lovvorn and Smith (1943) demonstrated that longer intervals between defoliations partially decreased the negative effects of severe defoliation.

REFERENCES

Abendroth, L.J., Elmore, R.W., Ferguson, R.B., 2006. G06-1622 Soybean Inoculation : Applying the facts to your fields (Part two of a two-part series). http://digitalcommons.unl.edu/cgi/viewcontent.cgi?article=3081&context=extensionhist (Accessed 1 Dec. 2015).

Adebisi, A.A., Bosch, C.H., 2004. *Lablab purpureus* (L.) Sweet. In Grubben, G.J.H, Denton O.A., (Eds.): Plant Resources of Tropical Africa (PROTA), no. 2, vegetables. PROTA Foundation, Wageningen, The Netherlands / Backhuys, Leiden, The Netherlands / CTA, Wageningen, The Netherlands, pp 343–348.

Abdul-Baki, A.A., Teasdale, J.R., 1995. Snap bean production in conventional tillage and in no-till hairy vetch mulch. Hort Sci. 33, 338-340.

Agishi, E.C., 1991. A bibliography on *Lablab purpureus*.Plant Science Division Working Document A6, International Livestock Centre for Africa (ILCA), Addis Ababa, Ethiopia. pp 76.

Agza, B., Binyam, K., Solomon, Z., Eskinde, A., Ferede, A., 2012. Animal feed potential and adaptability of some cowpea (*Vigna unguiculata*) varieties in northwest lowlands of Ethiopia. J. Agric. Res. 11, 478 – 483.

Akubdabweni, L.S., Peter-Paul, C., Singh, B.B., 1989. Evaluation of elite lines of cowpea (*Vigna unguiculata* [L.] Walp.) for leaf/fodder plus grain (i.e., dual-purpose). Trop. Agric. 67, 133-136.

Allen, D.J., Edje, O.T., 1990. Common bean in Africa farming systems. Progress in the improvement of common bean in Eastern and Southern Africa. African workshop series.

Amarger, N., Lobreau, J.P., 1982. Quntitative study of nodulation competitiveness in Rhizobium strains. Appl. Environ. Microb. 44, 583–588.

Anderson, J.R., 1978. Pesticide effects on non-target soil microorganisms. In Hill, I.R., Wright, S.J.L., (Eds.). pp 313-533.

Andrés J.A., Correa, N.S., Rosas, S.B., 1998. Survival and symbiotic properties of *Bradyrhizobium japonicum* in the presence of thiram: Isolation of fungicide resistant strains. Biol. Fertil. Soils. 26, 141-145.

Anten, N.P.R., Ackerly, D.D., 2001. Canopy-level photosynthetic compensation after defoliation in a Tropical understorey palm. Funct. Ecol. 15, 252–262.

Aryeetey, A.M., Laing, E., 1973. Inheritance of yield components and their correlation with yield in cowpea (*Vigna unguiculata* L. Walp.). Euphytica. 22, 386-392.

Mark, A., Livezey, J., Dohlman, E., 2006. Soybean Backgrounder. http/.ers.usda.gov/media/862892/ocs200601. (Accessed 1 Dec 2015).

Atkins, C.A., Kuo, J., Pate, J.S., Flinn A.M., Steele, T.W., 1977. Photosynthetic pod wall of pea (*Pisum sativum* L.), distribution of carbon-fixing enzymes in relation (*Pisum sativum* L.), distribution of carbon-fixing enzymes in relation to pod structure. Physiol. 60, 779-786.

Athar, M., Johnson, D.A., 1996. Influence of drought on competition between selected *Rhizobium meliloti* strains and naturalized soil rhizobia in alfalfa. Plant. Soil. 184, 231–241.

Awolumatea, E., 1983. Accumulation and quality of storage protein in developing cowpea , mung bean and soya bean seeds. J. Sci. Food. Agri. 34:1351–1357.

Awonaike, K.O., Kumarasinghe, K.S., Danso, S.K.A., 1990. Nitrogen fixation and yield of cowpea (*Vigna unguiculata*) as influenced by cultivar and *Bradyrhizobium* strain. Field Crops Res. 24, 163–171.

Badi, S.H., Dikwahal, H.D., Jibung, G.G., 2012. Response of Vegetable Cowpea (*Vigna unguiculata*) to Intra-Row Spacing and Defoliation at Garkawa. Asian J. Agric. Sci. 4, 210–212.

Balasubraminan, T., Sadasivam, S., 1987. Practical Manual in Biochemistry, Tamil Nadu Agricultural University, Coimbatore, India, p.14.

Baloyi, B.M., Ayodele, V.I., 2013. Effects of leaf harvest on crude protein and mineral contents of selected early maturing lines of lablab (*Lablab purpureus*). Afr. J. Agric. Res. 8, 449–453.

Barrett, R.P., 1987. Integrating leaf and seed production strategies for cowpea (*Vigna unguiculata* [L.] Walp). MS thesis. Michigan State University, East Lansing, MI, USA.

Bayne, H.G., Brown, M.S., Bethlenfalvay, G.J., 1984. Defoliation effects on mycorrhizal colonization , nitrogen fixation and photosynthesis in the *Glyicne-Glomus-Rhizobium* symbiosis. Physiol. Plant. 62, 576–580.

Begun, A., Eden, W.G., 1965. Influence of defoliation on yield and quality of soybeans. J. Econ. Entomol. 58, 591-592.

Berner, D.K., Kling, J.G., Singh, B.B., 1995. Striga research and control. A perspective from Africa. Plant Dis. 79, 652–660.

Beuerlein, J., 2007. Fungicide treated soybean seed makes more yield. http://www.greatlakeshybrids.com/posts/1773-osu-fungicide-treated-soybean-seed-makes-more-yield-jim-beuerlein. (Accessed 1 Dec. 2015).

Bhuvaneswari, T.V., Lesniak, A.P., Bauer, W.D., 1988. Efficiency of nodule initiation in cowpea and soybean. Plant. Physiol. 86, 1210–1215.

Bikrol, A., Saxena, N., Singh, K., 2005. Response of *Glycine max* in relation to nitrogen fixation as influenced by fungicide seed treatment. Afr. J. Biotechnol. 4, 667–671.

Bittenbender, H.C., Barrett, R.P., Indire-Lavusa, B.M., 1984. Beans and cowpeas as leaf vegetables and grain legumes. Bean/Cowpea CRSP occasional monograph No. 1. Bean/Cowpea CRSP, Michigan State University, East Lansing, MI, USA.

Blount, A.R.S., Wright, D.L., Sprenkel, R.K., Hewitt, T.D., Myer, R.O., 2009. Forage soybeans for grazing, hay and silage. University of Florida IFAS Extension. Publication #SS-AGR-1801,1-5. https://edis.ifas.ufl.edu/ag184 (accessed 30 Nov. 2015).

Board, J.E., Wier, A.T., Boethel, D.J., 1994. Soybean yield reductions caused by defoliation during mid to late seed filling. Agron. J. 86, 1074–1079.

Boyer, J.S., 1980. Physiological adaptations to water stress. Turner, N.C., and Kramer, P.J. (Eds.). Adaptations of plants to water and high temperature stress. J. Wiley, New York, NY. pp 443–444.

Bressani, R., 1985. Nutritive value of cowpea. Singh, S.R, and Rachie, K.O. (Eds.). Cowpea Research Production and Utilization. John Wiley and Sons, Chichester, London. pp. 353-356.

Brkić, S., Milaković, Z., Kristek, A., Antunović, M., 1995. Pea yield and its quality depending on inoculation , nitrogen and molybdenum fertilization. Plant Soil. Environ. 50, 39–45.

Brockwell, J., Bottomley, P.J., Thies, J.E., 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: a critical assessment. Plant. Soil. 174,143–180.

Browde, J.A., Pedigo, L.E., Owen, M.D.K., Tylka, G.L., Levene, B.C., 1994. Growth of soybean stressed by nematodes , herbicides , and simulated insect defoliation. Agron. J. 86, 968–974.

Bryant, J.P., Chapin, F.S., Klein, D.R., 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos. 40, 357–368.

Bubenheim, D.L., Mitchell, C.A., 1990. Cowpea harvest strategies and yield effective for space food production. HortScience. 23, 106.

Buruchara, R.A., 1990. Preliminary information on seed borne fungi of beans (*Phaseolus vulgaris*) in Kenya. In Proceedings of the second workshop on bean research in East Africa. CIAT Africa workshop series No. 7, Nairobi, Kenya. Pp 257-269.

Burns, R.C., Hardy, R.W.F., 1975. Nitrogen fixation in bacteria and higher plants. J. Basic Microbiol. 18, 152-153.

Burton, J.W., Israel, D.W., Wilson, R.F., Carter, T.E., 1994. Effects of defoliation on seed protein concentration in normal and high protein lines of soybean. Plant. Soil. 172, 131–139.

Bushby, H.V.A., 1984. Colonization of rhizospheres and nodulation of two *Vigna* species by rhizobia inoculated onto seed: influence of soil. Soil Biol. Biochem. 16, 635–641.

Cadwell, B.E., 1966. Inheritance of astrain specific ineffective nodulation in soybeans. Crop Sci. 6, 427-428.

Cameron, D.G., 1988. Tropical and subtropical pasture legumes. Queensland Agric. J. 114, 110–113.

Campo, R.J., Hungria, M., 2000. Compatibility use of fungicides in the treatment and inoculation of soybean seeds. http./www.agencia.cnptia.embrapa.br/Repositorio/inoculante.tratamento.sementes_000fltw5mca02wyiv80kxlb36i7uwrd1. (Accessed 1 Dec. 2015).

Cardinali, A., Linsalata, V., Perriono, P., Lattanzio, V., Brouillard, R., Jay, M., Scalbert, A., 1995. Chemotaxonomy of wild *Vigna* species as potential sources of resistance to insects. Polyphenols 94: 17th International Conference, Palma de Mallorca, Spain. pp 375–376.

Carsky R.J., Abaidoo, R.K., Dashiell, C., Sanginga, N., 1997. Effect of soybean on subsequent maize grain yield in the Guinea Savannah zone of West Africa. Afr. Crop Sci. J. 5, 31-38.

Catroux, G., 1991. Inoculant quality standards and controls in France. Thompson, J.A., (Eds.), Expert consultation on legume inoculant production and quality control, FAO, Rome, pp. 113–120.

Cisse, N., Ndiaye, M., Thiaw, S., Hall, A.E, 1995. Registration of Mouride cowpea. Crop Sci. J. 35, 1215-1216.

Conley, S., 2015. Characterising soybean yield respones to rhizobial inoculants. http://portal.nifa.usda.gov/web/crisprojectpages/0218132-characterizing-soybean-yield-response-to-rhizobial-inoculants.html. (Accessed 1 Dec. 2015).

Corriher-olson, V., Smith, G.R., 2013. Establishment of tropical annual legumes sod-seeded into bermudagrass or prepared seedbed. Tex. J. Agri. Nat. Resourc. 26, 73–81.

Chemining'wa, G.N., Muthomi, J.W., Obudho, E.O., 2004. Effect of rhizobia inoculation and urea application on nodulation and dry matter accumulation of green manure legume at Katumani and Kabete sites of Kenya.Legume Res. Network Project Newslett. Issue No.11. pp 13-17.

Chui, J.N., Keter, J.K.A., 2003. Effects of nitrogen fertilizer and bean (*Phaseolus vulgaris* L.) residue on yields of beans in different cropping systems. East Afr. Agric. For. J. 67, 37-46.

Craufurd, P.Q., Summerfield, R.J., Ellis, R.H., Roberts, E.H., 1997. Photoperiod, temperature and the growth and development of cowpea (*Vigna unguiculata*). In: Singh, B.B., Mohan Raj, D.R., Dashiell, K.E., Jackai, L.E.N. (Eds.) Advances in Cowpea Research. International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural Sciences (JIRCAS). UK, pp 75–86.

Cregan, P.B., Keyser, H.H., 1986. Host restriction of nodulation by *Bradrhizobium japonicum* Strain USDA 123 in soybean. Crop Sci. 26, 911-916.

Danso, S.K.A., Owiredu, J.D., 1988. Competitiveness of introduced and indigenous cowpea *Bradyrhizobium* strains for nodule formation on cowpeas [*Vigna unguiculata* (L) Walp.] in three soils. Soil Biol. Biochem. 20, 305–310.

Daramola, D.S., Danso, S.K.A., Hardarson, G., 1994. Nodulation, N_2 fixation and dry matter yeld in soybean (*Glycine Max* (L.) Merrill) inoculated with combinations of effective and ineffective *Bradyrhizobium japonicum* strains. Biol. Biochem. 26, 883-889.

Debra, S.K., Defoer, T., Bengaly, M., 1998. Integrating farmers' knowledge, attitude and practice in the development of sustainable Striga control interventions in southern Mali. Neth. J. Agric. Sci. 46, 65–75.

Deka, R.K.,Sarkar, C.R., 1990. Nutrient composition and anti-nutritional factors of *Dolichos* lablab seeds. Food Chem. 38, 239-246.

Demooy, B.E., Demooy, C.J., 1989. Effects of leaf-harvesting practices on yield and yield components of ER-7 cowpea (*Vigna unguiculata*) in semi-arid Botswana. Field Crops. Res. 22, 27–31.

Denison, R.F., Kiers, E.T., 2004. Lifestyle alternatives for rhizobia: mutualism, parasitism, and forgoing symbiosis. FEMS Microbiol. Lett. 237, 187–193.

Deshmukh, D.V, Mate, S.N., Bharud, R.W., Harer, P.N., 2011. Analysis of pod and seed development in cowpea [*Vigna unguiculata* (L .) Walp]. Am. Eurasian. J. Agron. 4, 50–56.

Dobereiner, J., Urguiaga, S., Boddey, R.M., 1995. Alternatives for nitrogen nutrition of crops in tropical agriculture. Fertil. Res. 42, 339–346.

Doung, V.C., Lan, P.T.P., Nguyen, T.T., Luu, H.M., Tu, B.T., 1984. Symbiotic nitrogen fixing efficiency of different rhizobial strains on grain and vegetable soybeans at Omon , Vietnam. http://www.researchgate.net/publication/237554401_Simbiotic_nitrogen_fixing_efficiency_of_different_rhizpbial_strains_on_grain_and_vegetable_soybean_at_Omon_Vietnam. (Accessed 1 Dec. 2015).

Drabo, I., Redden, R., Smithson, J.B., Aggarwal, V.D., 1973. Inheritance of seed size in cowpea (*Vigna unguiculata* (L.) Walp.). Euphytica. 33: 929–934.

Dunigan, E.P., Frey, J.P., Allen, L.D., McMahon, A., 1972. Herbicidal effects on the nodulation of *Glycine max* (L.) Merrill. Agron. J. 64, 806-808.

Eaglesham, A.R.J., Minchin, F.R., Summerfield, R.J., Dart, P.J., Huxley, P.A., Day, J.M., 1977. Nitrogen nutrition of cowpea (*Vigna unguiculata*). Distribution of nitrogen within effectively nodulated plants. Exp. Agric. 13, 369-380.

Eckel, C.S, Bradley, J.R.,Van-Duyn, J.W., 1992. Reductions in soybean yield and quality from corn earworm flower feeding. Agron. J. 68, 653-657.

Ehlers, J.D., Hall, A.E., 1997. Cowpea (*Vigna unguiculata* (L.) Walp.). Field Crops Res. 53, 187–204.

Elowad, H.O.A., Hall, A.E., 1987. Influences of early and late nitrogen fertilization on yield and nitrogen fixation of cowpea under well-watered and dry field conditions. Field Crops Res. 15, 229–244.

El-Shatnawi, M.J., Al-Qurran, L.Z., Ereifej, K.I., Turk, M., 2003. Defoliation of wall barley under sub-humid Mediterranean conditions. Aust. J. Agric. Res. 54, 53-58.

Entz, P., 1998. Managing crop inputs in pea production. In 20th Annual Manitoba-North Dakota Zero Tillage Workshop, “Zero Tillage and Farming’s Future”. Minot, ND. pp. 51–55.

Enyi, B.A.C., 1975. Effects of defoliation on growth and yield in groundnut (*Arachis hypogaea* L.), cowpeas (*Vigna Unguiculata*), soybean (*Glycine max*) and green gram (*Vigna aurens*). An. Appl. Biol. 79, 55-66.

FAO., 2015. *Lablab purpureus* (L.) Sweet.
http://www.fao.org/ag/agp/agpc/doc/gbase/data/pf000047.htm (Accessed 1 Dec. 2015).

FAO., I984. Legume inoculants and their use.
http./ctahr.hawaii.edu/bnf/Downloads/Training/Legume%20use/Title. (Accesse 1 Dec. 2015).

Fehr, W.R., Caviness, C.E., Burmood, D.T., Pennington, J.S., 1971. Stage of development descriptions for soybeans *Glycine max* (L.) Merrill. Crop Sci. 11, 929-931.

Fening, J., Danso, S.K., 2002. Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. Appl. Soil. Ecol. 21, 23–29.

Fery, R.L., 1990. The cowpea production, utilization, and research in the United States. Hort. Rev. 12,197–222.

Figueiredo, M.V.B., Vilar, J.J., Burity, H.A., de França, F.P., 1999. Alleviation of water stress effects in cowpea by *Bradyrhizobium* spp . inoculation. Plant. Soil. 207, 67–75..

Floor, J., 1985. Effect of soil fertility status, moisture and application of fertilizers and inoculum on nodulation and growth of dry beans in Kenya: In Ssali, H. and Keya, S.O (Eds) Biological nitrogen fixation in Africa.Matianum Press Consultants, Nairobi, pp 253-261.

Flynn, R. and J. Idowu. 2015. Nitrogen fixation by legumes.
http://aces.nmsu.edu/pubs/_a/A129/ (Accessed 30 Nov. 2015).

Freire, J.R.J., 1982. Research into the *Rhizobium-Leguminosae* symbiosis in Latin America. Plant. Soil. 67, 227-239.

Fujita, K., Otsu, K.G., Ogta, S., 1992. Biological nitrogen fixation in mixed legume-cereals cropping systems. Plant. Soil. 141, 155-175.

Gaur, A.C., 1980. Effect of pesticides on symbiotic nitrogen fixation by legumes. J. Microbiol. 20, 362-370.

Gazzoni, C.I.O.L., Moscardi, V.I.O., 1997. Effect of defoliation levels on recovery of leaf area , on yield and agronomic traits of soybeans. Braz. J. Agric. Res. 33, 411-424.

Gentili, F., Huss- Danell, K., 2003. Local and systemic effects of phosphourous and nitrogen on nodulation and nodule formatting in *Alnus incana*. J. Exp. Bot. 54, 2757-2767.

Gentili, F., Huss- Danell, K., 2002. Phosphorus modifies the effects of nitrogen nodulation in split-rot systems of hippophea. New Phytol. 153, 53-61.

Gibson, L. R., & Mullen, R. E. (1996). Influence of day and night temperature on soybean seed yield. *Crop Science*, *36*(1), 98–104.

Giller, K.E., 2001. Nitrogen fixation in tropical cropping systems, 2nd Edition. http://www.researchgate.net/publication/40159031_Nitrogen_Fixation_in_Tropical_Cropping_Systems. (Accessed 1 Dec. 2015).

González, E.M., Gordon, A.J., James, C.L., Arrese-Igor, C., 1995. The role of sucrose synthase in the response of soybean nodules to drought. J. Exp. Bot. 291, 1515–1523.

Gould, G.E., 1960. The effect of Japanese beetle feeding on the yield of soybeans. Proc. Indiana Acad. Sci. 69, 178-191.

Goring, C.A.I., Laskowski, D.A., 1983. The effects of pesticides on nitrogen transformations in soils. Agron. Monogr. 22, 689-720.

Guerin, V., Pladys, D., Trinchant, C., Rigaud, J., 1991. Proteolysis and nitrogen fixation in faba-bean (*Vicia faba*) nodules under water stress. Physiol. Plant. 82, 360–366.

Gutteridge, J.M.C., Richmond, R.R.I.C., Halliwell, B.H., 1980. Oxygen free-radicals and lipid peroxidation : Caeruloplasmin inhibition by the protein. FEBS. Lett. 112, 269–272.

Graham, P.H., 1992. Stress tolerance in rhizobium and *bradyrhizobium* and nodulation under adverse soil conditions. Can. J. Microbiol. 38, 475-484.

Greenfield, P.L., 1991. The influence of method of inoculation and certain herbicides on nodulation and seed yield of soybeans. S. Afr. J. Plant Soil. 8, 119-122.

Grymes, C.F., Griffin, J.L., Boethel, D.J., Leonard, B.R., David, L., Russin, J.S., Jordan, D.L., 1999. Soybean response to weed interference and defoliation. Weed Sci. 47, 90–94.

Hall, A.E., 2004. Breeding for resistance to drought and heat in cowpea. Eur. J. Agron. 21, 447-454.

Halverson, L.J., Stacey, G., 1986. Signal exchange in plant microbe interactions. Microbiol. Rev. 50, 193-225.

Ham, G.E., Caldwell, V.B., Johnson, H.W., 1971. Evaluation of *Bradyrhizobium japonicum* in soils containing naturalized populations of Rhizobia. Agron. J. 63, 301- 303.

Hammond, B., 1988. Effects of leaf removal at soybean growth stage V1 on yield and other growth parameters. J. Kans. Entomol. Soc. 62, 96–102.

Harrison, H.F., Thies, J.A., Fery, R.L., Smith, J.P., 2006. Evaluation of cowpea genotypes for use as a cover crop. Hortsci. 41, 1145–1148.

Hardarson, G., Gareth Jones, D., 1979b. The inheritance of preference for strains of Rhizobium trifolii by white clover (*Trifolium repens*). Ann. Appl. Biol., 92, 329-333.

Hans, N.E., Kingman, S.M., 1990. Dietary biber and resistant starch. A nutritional classification of plant polysaccharides. http://link.springer.com/chapter/10.1007%2F978-1-4613-0519-4_4#page-1. (Accessed 1 Dec. 2015).

Hashem, F.M., Saleh, S.A., Berkum, P.V., Voll, M., 1997. Survival of *Bradyrhizobium* spp. (*Arachis hypogaea* L.) on fungicide-treated peanut seed in relationship to plant growth and yield. World J. Microbiol. Biotechnol. 13, 335–340.

Henriet, J., Van Ek, G.A., Blade, S.F., Singh, B.B., 1997. Quantitative assessment of traditional cropping systems in the Sudan savanna of northern Nigeria. I. Rapid survey of prevalent cropping systems. Sam. J. Agric. Res. 14, 37–45.

Higley, L.G., 1992. New understandings of soybean defoliation and their implications for pest management. L.G. Copping (Eds.) Pest management in soybean. pp. 56–65.

Hikosaka, K., Takashima, T., Kabeya, D., Hirose, T., Kamata, N., 2005. Biomass allocation and leaf chemical defence in defoliated seedlings of *Quercus serrata* with respect to carbon-nitrogen balance. Ann. Bot. 95, 1025–1032.

Hintz, R.W., Beeghly, H.H., Fehr, W.R., Schneiter, A.A., Hicks, D.R., 1991. Soybean response to stem cutoff and defoliation during vegetative development. J. Prod. Agric. 4, 585–589.

Hirsch, P.R., 1996. Population dynamics of indigenous and genetically modified rhizobia in the field. New Phytol. 133, 159–171.

Hoogesteger, J., Karlsson, P.S., 1992. Effects of defoliation on radial stem growth and photosynthesis in the mountain birch (*Betula pubescens* spp. tortuosa). Funct. Ecol. 6, 317–323.

Howle, P.K.W., Snipe, E.R., Skipper, H.D., 1987.Soybean specificity for *Bradyrhizobium japonicum* strain 110. Agron. J. 79, 595-598.

Hudgens, R.E., 1996. Sustaining soil fertility in Africa: The potential for green manures. A paper for 15th Conference of the Soil Society of East Africa (SSEA) 19-23 August 1996, Nanyuki, Kenya.

Humphreys, L.R. 1994. Tropical forages: Their role in sustainable agriculture. Longman scientific and technical. pp. 414.

Imungi, J.K., Potter, N.N., 1983. Nutrient con- tents of raw and cooked cowpea leaves. J. Food Sci. 48, 1252–1254.

Jaworski, E.G., 1971. Nitrate reductase assay in intact plant tissues. Biochem. Biophys. Res. Commun. 43, 1274–1279.

Johnston, T. J., Pendleton, J.W., 1968. Contribution of leaves at different canopy levels to seed production of upright and lodged soybeans (*Glycine max* (L.) Merrill). Crop Sci. 8, 291-292.

Kandaswami, C., Middleton, E.Jr., 1994. Free radical scavenging and antioxidant activity of plant flavonoids. Adv. Exp. Med. Biol. 366, 351-376.

Kang, B.T., 1975. Effect of inoculation and nitrogen fertilizer on soybean in Western Nigeria. Exp. Agr. 11, 23-31.

Kálalová, S., Šimon, T., 1993. Efficiency of commercial inoculants for soybean. Rostl. Výr. 39, 827–834.

Karikari, S.K., and G. Molatakgosi. 1999. Response of cowpea [Vigna unguiculata (L.) Walp.] varieties to leaf harvesting in Botswana. UNI. J. Agric. 8, 5–11.

Karachi. M., 1997. Growth and nutritive value of *Lablab purpureus* accessions in semi-arid Kenya. Trop Grassl. 31, 214–218.

Kudo, G., 1996. Herbivory pattern and induced responses to simulated herb- ivory in *Quercus mongolica* var. *grosseserrata*. Ecol. Res. 11, 283–289.

Kundu, G.G., Trimohan., 1989. Effect of Rhizobium in association with granular insecticides on nodulation and yield in Soybean. Curr. Sci. 58, 1340-1342.

Klubertanz, T. H., Pedigo, L.P., Carlson, R.E., 1996. Soybean physiology, regrowth, and senescence in response to defoliation. Agron. J. 88, 577–582.

Kutcher, H.R., Lafond, G., Johnston, A.M., Miller, P.R., Gill, K.S., May, W.E., 2002. Rhizobium inoculant and seed-applied fungicide effects on field pea production. Can. J. Plant. Sci. 82, 645–661.

Kwapata, M.B., Hall, A.E., 1990a. Response of contrasting vegetable-cowpea cultivars to plant density and harvesting of young green pods 1. Pod production. Field Crops Res. 24, 1–10.

Kwapata, M.B., Hall, A.E., 1990b. Determinants of cowpea (*Vigna unguiculata*) seed yield at extremely high plant density. Field Crops Res. 24, 23–32.

Law, I.J., Botha, W.F., Majaule, U.C., Phalane, F.L., 2006. Symbiotic and genomic diversity of "cowpea" bradyrhizobia from soils in Botswana and South Africa. Biol. Fertil. Soils. 43, 653–663.

Laws, T. & Graves, W.R. 2005. Nitrogen inhibits nodulation and reversibly suppresses nitrogen fixation in nodules of Alnus maritima Journal of American Horticultural Science 130, 496-499.

Lawn, R.J., Brun, W.A., 1974. Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source sink manipulations. Crop Sci., 14, 11-16.

Layzell, D.B., Rainbird, R.M., Atkins, C.A., Pate, J.S., 1979. Economy of photosynthate use in nitrogen-fixing legume nodules. Plant Physiol. 64, 888–891.

Ligon, L.L., 1958. Characteristics of Cowpea varieties: *Vigna sinensis*. Oklahoma State Univ. Bull. B-518, pp.47.

Lovvorn, R. L., Smith, B.E.W., 1943. Response of soybeans to experimental defoliation. J. Am. Soc. Agron. 35, 768–778.

Maass, B.L., M.R. Knox, S.C. Venkatesha, T.T. Angessa, S. Ramme and B.C. Pengelly. 2010. Lablab purpureus-A Crop Lost for Africa? Trop. Plant. Biol. 3, 123–135.

Mallarino, A., Sawyer, J.E., Barnhart, S.K., 2013. A general guide for crop nutrient and limestone recommendations in Iowa.

Martins, L.M.V., G.R. Xavier, F.W. Rangel, J.R.A. Ribeiro, M.C.P. Neves, L.B. Morgado, and N.G. Rumjanek. 2003. Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. Biol. Fertil. Soils. 38:333–339.

Mcalister, D.F., and O.A. Krober. 1958. Response of soybeans to leaf and pod removal. Agron. J. 50, 674–677.

McWatters, K.H., Chhinnan, M.S., 1985. Effect of hydration of cowpea meal on physical and sensory attributes of a traditional West African food. J. Food Sci. 50, 444-446.

Midega, C.A.O., D. Salifu, T.J. Bruce, J. Pittchar, J.A. Pickett, and Z.R. Khan. 2014. Cumulative effects and economic benefits of intercropping maize with food legumes on *Striga hermonthica* infestation. Field Crops. Res. 155, 144–152.

Miller-Cebert, R.L., Sistani, N.A., Cebert, E., 2009. Comparative protein and foliate content among canola cultivars and other cruciferous leafy greens. J. Food Agric. Environ. 7, 46-49.

Minson, D.J., Milford, R., 1967. The voluntary intake and digestibility of diets containing different proportions of legume and mature pangola grass (*Digitaria decumbens*). Aust. J. Exp. Agric. Anim. Husb. 7, 546-551.

Mohan, N., Aghora, T.S., 2006. Collection and evaluation of Dolichos bean (*Lablab purpureus* [L.] Sweet) Germplasm in Tamil Nadu, India. Poster presented at Int. Conf. on Indigenous Vegetables and Legumes: Prospects for Fighting Poverty, Hunger and Malnutrition. 12–15 Dec. 2006, Patancheru, India.

Mpepereki, S., Wollum, A.G., Makonese, F., 1996. Diversity in symbiotic specificity of cowpea rhizobia indigenous to Zimbabwean soils. Plant. Soil. 186, 167–171.

Muir, J.P., Reed, L.R., Malinowski, D.P., 2005. Impact of defoliation on herbage and seed production of *Strophostyles helvula* and *S. leiosperma.* Nat. Plants J. 6, 123–130.

Murphy, A.M., Colucci, P.E., 1999. A tropical forage solution to poor quality diets: A review of *Lablab purpureus*. Livestock Research for Rural Development. http://www.fao.org/livestock/agap/frg/lrrd/lrrd11/2/colu112.htm. (Accessed 1 Dec. 2015).

Murtagh, G.J., Dougherty, A.B., 1968. Relative yields of lablab and velvet bean. Trop Grassl. 2, 57-63.

Muthomi, J.W., Otieno, P.E., Chemining, G.N., Nderitu, J.H., 2007. Effect of root rot pathogens and fungicide seed treatment on nodulation in food grain legumes. J. Biol. Sci. 7, 1163–1170.

Niemann, S., Pühler, A., Tichy, H.V., Simon, R., Selbitschka,W., 1997. Evaluation of the resolving power of three different DNA finger- printing methods to discriminate among isolates of a natural Rhizobium meliloti population. J. Appl. Microbiol. 82, 477–484.

Neves, M.C.P., 1981. Physiological interdependence between the componets of the symbiotic system. Rhizobium-legume.Interdependência fisiológica entre os compo- nentes do sistema simbiótico Rhizobium-leguminosas. Braz. Soc. Soil. Sci. 5, 79–92.

Ng, Q., Padulosi, S., 1988. Cowpea gene pool distribution and crop improvement. Ng, Q., Perrino, P., Attere, F., Zedan, H. (Eds.) Crop Genetic Resources of Africa, Vol II. IBPGR, Rome, pp 161–174.

Norton, B.W., Poppi, D.P., 1995. Composition and nutritional attributes of pasture legumes. Tropical legumes in animal nutrition. D'Mello, J.P.F., Devendra, C. (Eds.), CAB International. Wallingford, UK. pp 45-60.

Nowak, R.S., Caldwell, M.M., 1984. Atest of compensatory photosynthesis in the field: implications for herbivory tolerance. Oecologia. 61, 311–318.

Nyambati, E.M., Sollenberger, L.E., Hiebsch, C.K., Sylvia, D.M., Rono, S.C., 2001. Nitrogen contribution from relay-cropped mucuna and lablab to succeeding maize in the sub-humid highlands of Kenya. Agron. Abstr. CD-ROM, a06- sollenberger142348-0.

Ogedegbe S.A., Ongunlela, V.B., Olufajo, O.O., Odion, E.C., 2012. Seed yield and yield attributes of lablab as influenced by phosphorous application, cutting height. Asian. J. Crop. Sci. 4, 12–22.

Ohler, T.A., Nielsen, S.S., Mitchell, C.A., 1996. Varying plant density and harvest time to optimize cowpea leaf yield and nutrient content. Am. Soc. Hortic. Sci. 3, 193–197.

Osa-Afiana, L.O., Alexander, M., 1982. Clays and survival of Rhizobium in soil during desiccation. Soil Sci. Soc. Am. J. 46, 285–288.

Otieno, P.E., Muthomi, J.W., Chemining'wa, G.N., Nderitu, J.H., 2007. Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected food grain legumes. Afr. Crop. Sci. Soc. 8, 305–312.

Pasaribu, D., Sunarlim, N., Sumarno, Supriati, Y., Saraswati, R., Partohardjono, S., Karama, S., 1989. Rhizobium inoculation study in Indonesia. Central Research Institute for Food Crops, Bogor, Indonesia. pp. l-29.

Pengelly, B.C., Maass, B.L., 2001. *Lablab purpureus* (L.) Sweet—diversity, potential use and determination of a core collection of this multi- purpose tropical legume. Genet. Resour. Crop. Evol. 48, 261–272.

Pickle, C.S., Caviness, C.E.,1983.Yield reduction from defoliation and plant cutoff of determinate and semideterminate soybean. Agron. J. 4, 474–476.

Pimentel, C., Jacob, J.N., Goi, S.R., Pessanha, G.G., 1990. Water stress in *Phaseolus vulgaris* L. cultivars in symbiosis with Rhizobium leguminosarum biovar phaseoli. Turrialba. 40, 520–526.

Pueppke. S.G., 1992. Broad host range Rhizobium species strain NGR234 secretes a family of carbamoylated, and fucosylated, nodulation signals that are 0- acetylated or sulphated. Mol. Microbiol. 6, 3575-3584.

Rahman, S.A., Ibrahim, U., Ajoji, F.A., 2008. Effect of defoliation at different growth stages on yield and profitability of cowpea (*Vigna unguiculata* (L.)Walp.). Elec. J. Env. Agric. Food Chem. 7, 3248-3254.

Rao, S.C., Northup, B.K., 2009. Capabilities of four novel warm-season legumes in the southern great plains: Biomass and forage quality. Crop. Sci. 49, 1096–1102.

Rennie, R.J., and S. Dubetz. 1982. Effect of fungicides and herbicides on nodulation and N_2 fixation in soybean fields lacking indigenous *Rhizobium japonicum*. Agron. J. 76, 451–454.

Rhodes, E.R., Nangju, D., 1979. Effects of pelleting cowpea and soybean seed with fertilizer dusts. Exp. Agric. 15, 27-32.

Rotimi, A.O., 1972. Effect ofinoculation with commercial peat- based cowpea rhizobium strain on the developmentof cowpea varieties. Niger. Agric. J. 7, 74-179.

Rupela, O.P., Toomsan, B., Mittal, S., Dart, P.J., Thompson, J.A., 1987. Chickpea Rhizobium populations: Survey of influence of season, soil depth and cropping pattern. Soil Biol. Biochem. 19, 247-252.

Salisbury, F.B., Ross, C.W., 1992.Plant physiology (4th Ed.).Wadsworth, Belmont, California, p.184.

Schaaffhausen, R. V. (1962). Dolichos lablab or Hyacinth Bean : Its uses for feed, food and soil improvement. Econ. Bot. 17, 146-153.

Schulz, T.J., Thelen, K.D., 2008. Soybean seed inoculant and fungicidal seed treatment effects on soybean. Crop. Sci. 48, 1975–1983.

Schulz, T.J., Thelen, K.D., Wang, D., 2005. The effect of *Bradyrhizobium japonicum* inoculant on soybean growth and yield. http://www.researchgate.net/publication/237651647_The_Effect_of_*Bradyrhizobium_japonicum*_inoculant_on_soybean_growth_and_yield (Accessed 30 Nov. 2015).

Seneviratne, G., Van-Holm, L.H.J, Ekanayake, E.M.H.G.S., 2000. Agronomic benefits of rhizobial inoculant use over noitigen fertilizer application in Tropical soybean. Field Crops. Res. 68, 199-203.

Shehu,Y., Alhassan, W.S., Pal, U.R., Phillips, C.J.C., 2001. The effects of sowing date on the growth and nutritive value of *Lablab purpureus*. J. Agron. Crop Sci. 186, 21–29.

Singleton, P.W., Stockinger, K.R., 1983. Compensation against ineffective nodulation in soybean. Crop Sci. 25, 69-72.

Smith, R.S., 1992. Legume inoculant formulation and application. Can. J. Microbiol. 38, 485-492.

Somasegaran, P., Hoben, H.J., 1985. Methods in legume-*Rhizobium* technology. University of Hawaii NifTAL. Hawaii Institute of tropical agriculture and human resources.

Sparrow, S.D., Ham, G.E., 1983. Nodulation, N_2 fixation and yield of Navy beans as influenced by inoculant rate and inoculant carrier. Agron. J. 75, 20-24.

Stamford, N., Santos, D.R., Silva, V.M., Santos, C.E.R.S., Monteiro, M.C., 1990. N_2 fixation and dry matter of cowpea: Two Brazilian semiarid soils subjected to water deficit. Braz. Soc. Soil. Sci. 14, 283–290.

Steele, W.M., Mehra, K.L., 1980. Structure, evolution and adaptation to farming systems and environments in *Vigna* . Summerfield, R.J., Bunting, A.H., (Eds.). Advance in legume science. pp. 393-404.

Streeter, J.G., 1993. Translocation. A key factor limiting the efficiency of nitrogen fixation in legume nodules. Physiol. Plant. 87, 616–623.

Strijdom, B.W., 1998. South African studies on biological nitrogen fixing systems and the exploitation of the nodule bacterium legume symbiosis. S. Afr. J. Sci. 94, 11–23.

Strijdom, B.W., Otto, C.J., Lochner, H.H., 1988. Effects of inoculant strains applied over two seasons on nodulation of groundnuts by indigenous rhizobia. S. Afr. J. Sci. 84, 115–118.

Taylor, S.R., Weaver, B.D., Wood, W.C., Santen, V.E., 2005. Nitrogen Application increases yield and early dry matter accumulation in late-planted soybean. Crop Sci. J. 45, 854-858.

Teasdale, J.R., Shirley, D.W., 1998. Influence of herbicide application timing on corn production in a hairy vetch cover crop. J. Prod. Agr. 11, 121-125.

Teigen, J.B., Vorst, J.J., 1975. Soybean response to stand reduction and defoliation. Agron. J. 67, 813–816.

Thies, J.E., Singleton, P.W., Bohlool, B.B., 1995. Phenology, growth, and yield of field-grown soybean and bush bean as a function of varying modes of N nutrition. Soil Biol. Biochem. 27, 575–583.

Thombre, P.A., Kurundkar,B.P., Kawla, B.R., 1989. Effect of fungicidal seed treatments on nodulation and yield of soybean. J. Oilseeds Res. 6, 353-356.

Todd, J.W., Morgan, L.W., 1972. Effect of hand defoliation on yield and seed weight of soybeans. J. Econ. Entomol. 65, 567-570.

Toro, A., 1996. Nodulation competitiveness in the *Rhizobium*-legume symbiosis. World. J. Microbiol. *Biotechnol*. 12, 157-162.

Tuomi, J., Niemela, P., Haukioja, E., Sire, S., Neuvonen, S., 1984. Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. Oecologia 61, 208–210.

Tuomi, J., Niemela, P., Sire, S., 1990. The panglossian paradigm and delayed inducible accumulation of foliar phenolics in mountain birch.Oikos. 59, 399–410.

Trang, K.M., Giddens, J., 1980. Shading and temperature as environmental factors affecting growth, nodulation and symbiotic N_2 fixation by soybeans. Agron. J. 72, 305-308.

Turnipseed, S.G., 1972. Response of soybeans to foliage losses in South Carolina. J. Econ. Entomol. 65, 224-229.

Van Kessel, C., Hartley, C., 2000. Agricultural management of grain legumes:has it led to an increase in nitrogen fixation? Field Crops Res. 65, 165–181.

Van Schaik, P.H., Probst, A.H., 1958. Effects of some environmental factors on flower production and reproductive efficiency in soybeans.1. Agron. J. 50, 192-197.

Vance, C.P. 2001. Symbiotic nitrogen fixation and Phosphorous acquisition. Plant nutrition in a world declining renewable resources. Plant. Physiol. 127, 390- 397.

Venkateswarlu, B., Maheswari, M., Saharan, N., 1989. Effects of water deficit on N_2(C2H2) fixation in cowpea and groundnut. Plant Soil. 114, 69–74.

Walker, D.W., Miller, C.J., 1986. Influence of water stress on nitrogen fixation in cowpea. J. Am. Soc. Hortic. Sci. 111, 451– 458.

Warrag, M.O.A., Hall, A.E., 1984. Reproductive responses of cowpea (*Vigna unguiculata* (L.) Walp.) to heat stress. Responses to night air temperature. Field Crops Res. 8, 17-33.

Whitbread, A.M. and Pengelly, B.C., eds., (2004) Tropical Legumes for Sustainable Farming Systems in Southern Africa and Australia. ACIAR Proceedings No. 115. ACIAR Canberra 180p. http://www.aciar.gov.au/publication/PR115. (Accessed 1 Dec. 2015).

Willmer, C.M., Johnston, W.R., 1976. Carbon dioxide assimilation in some aerial plant organs and tissues. Planta. 130, 33-37.

Wilson, J.B., 1988.Areview of evidenceonthe control of shoot : root ratio, in relation to models. Ann. Bot. 61, 433–449.

Wilson, G.P., Murtagh, G.J., 1962. Lablab: A new forage crop for the north coast. Agric. Gazette. N.S.W. 73, 460-462.

Wood, I.,1983. Lablab bean (*Lablab purpureus*) for grain and forage production in the Ord irrigation area. Aust. J. Exp. Agric. 23, 121-162.

Woomer, P., Singleton, P.W., and Bohlool, B.B., 1988). Ecological indicators of native rhizobia in tropical soils. Appl. Environ. Microbiol. 54, 1112-1116.

Xavier, G.R., Martins, L.M.V., Neves, M.C.P., Rumjanek, N.G., 1998. Edaphic factors as determinants for the distribution of intrinsic antibiotic resistance in a cowpea rhizobia population. Biol. Fertil. Soils. 27, 386–392.

CHAPTER 2

INFLUENCE OF *BRADYRHIZOBIUM* INOCULATION AND FUNGICIDE TREATMENT ON DEVELOPMENT AND YIELD OF SELECTED PULSE CROPS

A manuscript for submission to Agronomy Journal

Abstract

Declining soil fertility due to inadequate nitrogen in most soils limits farmers' goals of increasing yield. There is environmental and agricultural benefit of reducing dependence on nitrogen fertilizer by emphasizing adaptation of favorable farming practices that minimize nitrogen fertilizer. Cowpea [*Vigna unguiculata* (L.) Walp.] and lablab (*Lablab purpureus* L.) are pulses with potential for grain, forage, and cover crops in the Midwest of United States, but little is known about their growth, development, and productivity potential in Iowa. We conducted a two-year field study to determine the influence of rhizobia inoculation and fungicide seed treatment of selected pulses. The objectives of this study were to i) determine the influence of fungicide seed treatment and inoculation on nodulation and nitrogen fixation of four pulses; soybean '92Y82', cowpeas ('CA46' and 'Top Crop') and lablab 'Rongai' and ii) determine the effect of fungicide seed treatment and *Bradyrhizobium* spp. inoculation on grain yield and above-ground biomass of the crops. The experimental design was a randomized complete block in a factorial of four pulse crops with and without rhizobium inoculation and a fungicide seed treatment. Although several research studies indicated that fungicide seed treatment and inoculation with *Bradyrhizobium* affected nodulation, nitrogen fixation and yield components, we found no significant effects of fungicide seed treatment and *Bradyrhizobium* inoculation on pulse grain yield.

Fungicide treatment and rhizobial seed inoculation are common practices prior to planting pulse crops, but reports vary widely for their impact on emergence, growth, development, and yield. Inoculation is done to provide sufficient numbers of viable and effective rhizobia that will induce fast colonization of the rhizosphere to enable rapid nodulation after seed germination and produce optimum yields at harvest (Catroux, 1991). Inoculation in subsequent years may be unnecessary if a legume becomes well established and nodulated (Van-Kessel and Hartley, 2000). The combination of fungicide and bacterial inoculant seed applications has long been used to control root diseases in legumes and increase yield. Up to 40% yield increase was observed in soybean with *Bradyrhizobium japonicum* inoculation compared to non-inoculated soybean (Schulz et al., 2005). In regions like Africa where dry seed yields are greatly affected by diseases, low soil fertility, pests as well as poor weather conditions such as drought (Allen, 1990; Otsyula et al., 1998), the impact on yield of fungicide seed treatment and inoculation often has increased pulse seed yield. Commercial fertilizer is costly to farmers and in developing countries, small-scale farmers are further constrained by lack of access (Otieno et al., 2007). The need for rhizobial inoculation does not mean that rhizobia do not exist in the soils. The amount of rhizobia varies depending on type of soil, soil cultivation type as well as environment (Hirsch, 1996). Since rhizobial populations in bulk soil rarely exceed 10^6 nodulating cells g^{-1} soil, these are minor compared to the estimated $10^8 – 10^9$ viable bacterial cells g^{-1} of soil (Hirsch, 1996). Hirsch further reported that rhizobial numbers may reach higher numbers in the rhizosphere.

Biological nitrogen fixation offers an inexpensive alternative to meet much of the nitrogen needs for pulse crop production. The combination of high levels of N fixation with

better pest and disease management strategies from seed fungicide application may increase yield and ensure sustained food and nutrition security, especially in developing countries. Legumes form a symbiotic relationship with specific rhizobia bacteria and produce root nodules in response to infection. Bacteria in the root nodules have the potential to convert atmospheric nitrogen to forms usable by the host plant. Annually 44 to 66 million tons of nitrogen are fixed worldwide by legumes and this accounts for nearly half of all the nitrogen that is used in agriculture (Giller, 2001). Burns and Hardy (1975) reported that legumes symbiotically fixed about 175×10^6 tons of nitrogen per year worldwide, greatly decreasing fertilizer N demand. Many researchers reported that yields could be increased by legume seed inoculation compared to use of nitrogen fertilizer (Kalalova and Simon, 1993; Dobereiner et al., 1995). Although different legume species differ in the amount of nitrogen that they can fix and supply crop needs (Chui et al., 2003), symbiotic nitrogen fixation plays an important role in providing nitrogen to other non-fixing plants in intercrops (Fujita et al., 1992; Sanginga et al., 1994; Hudgens et al., 1996; Carsky et al., 2000). Symbiotic nitrogen fixation is important where non-fixing crops such as corn (*Zea mays* L.) follow a legume the next season. Farmers are often uncertain about the levels of indigenous rhizobia and its responsiveness to the legume crop, therefore inoculation with rhizobia is a cost-effective way to ensure that maximum yield is attained (Kutcher et al., 2002). Entz (1998) and Rice et al. (2000) reported that application of inoculant increased nodule number and did this result in greater yield or aboveground biomass of the pulse crop(s).

Inoculation of seeds is a common practice; however, rhizobia can also be directly applied to seed rows at planting. The latter results in better distribution of rhizobia in soil and the potential to form many nodules on both primary and lateral roots of the plant unlike seed inoculation where nodules can be more clustered on the crown and upper roots (Daramola et al.,

1994). Direct inoculation in the soil gives inoculated strains more capability to compete with indigenous naturally-occurring rhizobium strains in the soil (Danso and Owiredu, 1988). Rhizobium inoculation may be done using single, double or multiple strains (Daramola et al., 1994). However, since a particular rhizobium strain may be effective on one plant and not on another (Jones and Hardarson, 1979; FAO, 1984), using a mixed strain for inoculation may be the most effective means to compete against ineffective strains (Daramola et al., 1994).

Pulse production can be greatly influenced by root rot diseases (Muthomi et al., 2007), which are known to hinder seedling germination and cause post emergence damping off, leading to poor crop stands and low yields. Disease-causing pathogens can be soil-borne or seed-borne and farmer practices of seed storage and transfer negate vital sanitary principles (Buruchara, 1990). In many developing regions like Africa, it is common for farmers to share seeds among households as well as to save seeds from previous harvests for replanting. Sanitation and use of clean planting materials are recommended primary practices for preventing damping off and other root diseases; however, chemical seed treatment prior to planting is now a common practice.

The combination of rhizobial inoculation and seed fungicide treatment can be problematic. Muthomi et al. (2007) reported that sometimes the seed-applied fungicide suppressed the effectiveness of the rhizobia bacteria in lablab and common bean (*Phaseolus vulgaris* L.). Fungicide seed treatment limited soybean nodulation and subsequent grain yield (Andres et al., 1998; Campo and Hungria, 2000). Most seed fungicides can be toxic to rhizobia during prolonged exposure although inoculation and planting shortly within a few hours may have little ill effect on the inoculant (Van Kessel and Hartley, 2000). Hashem et al. (1997) and Thombre et al. (1989) reported that the compatibility of *Bradyrhizobium* and fungicides was

uncertain as the tested fungicides differed in their effects on the survival and growth of rhizobium strains in peanuts and soybean respectively. The effect of fungicide on the strains also depended on the strains and concentration of fungicide applied (Hashem et al., 1997). The formulation of inoculant impacted how fungicide affected nodulation in legumes (Kutcher et al., 2002). Dunigan et al. (1972) and Goring and Laskowski (1982) also reported that inhibition of rhizobia and nodulation by seed-applied fungicide in soybean arose from contact and concentration especially on primary roots although the permanent effects on nodulation of lateral roots were limited (Rennie and Dubetz, 1982). Fungicide and rhizobium inoculant combinations increased soybean yield in situations where fungicide had no impact on the inoculant (Schulz and Thelen, 2008). They reported that rhizobium inoculation yielded an average yield increase of 86.6 kg ha^{-1} from 14 site-years which had previous soybean [*Glycine max* (L.) Merr.] cropping history. With an average inoculant cost of \$8.5 ha^{-1}, they determined that annual soybean inoculation with fungicide seed treatment was cost effective to farmers. Bikrol et al. (2005) also reported that pretreatment of seeds with a fungicide had a positive role in biologically fixed nitrogen in soybean grain protein content increased by 10% on average with fungicide seed treatment and inoculation (Schulz and Thelen, 2008). However, they also reported a 3% decline in harvested yield with inoculant-fungicide combinations in one year.

Findings by Bikrol et al. (2005) showed a significant negative correlation between rhizobium inoculation-fungicide treatment and soybean plant protein content, contrary to Schulz and Thelen (2008). Bikrol et al. (2005) also noted that fungicide applied to soybean seeds may have affected the symbiotic relationship necessary for nitrogen fixation. The effect of fungicide on rhizobial establishment may also vary under different soil and climatic conditions. Gaur (1980) also reported that fungicide applied to another crop may be sufficiently persistent in the

soil at levels that are enough to affect nitrogen fixation as well as nitrogen levels in the soil. Cowpea [*Vigna unguiculata* (L.) Walp.] and lablab (*Lablab purpureus* (L.) could likely have similar problems. At a site in Montmorency, Michigan, Schulz and Thelen (2008) reported negative significant effects of fungicide on soybean yield in inoculated plots. From their research, a 500 kg ha^{-1} decrease in yield was observed in *Bradyrhizobium*-inoculated plots with seed-applied fungicide compared to inoculated subplots which did not receive fungicide. They observed no significant yield differences between uninoculated subplots with or without seed fungicide treatment. Further, the formulation of inoculant may influence the interaction between the inoculant and fungicide as well as nodulation, nitrogen fixation and yield. Although liquid inoculants are known to be more effective on soils where legumes have not been grown before, Schulz and Thelen (2008) reported that these advantages diminished when fungicide seed treatment was done with soybean. However, several other research studies, reported no differences following treatment of seeds with fungicide and inoculation. Treatment of soybean seeds with fungicide before and after sowing made no difference in nodule number or dry weight (Bikrol *et al*., 2005). Schulz and Thelen (2008) found seed-applied fungicide to provide no widespread yield improvement with inoculation. In sites where yield increment was observed, Schulz and Thelen (2008) ascertained that the yield increment was attributable to favorable climate such as temperature and rainfall and not inoculation. The researchers further reported that rainfall preceding planting may have caused more early soil-borne fungal diseases which were managed by the seed-applied fungicide hence increased yield, but not necessarily because of inoculation, following fungicide seed treatment. Nitrogen fixation by rhizobia in soybean appeared to increase with increase in soil temperatures up to 30°C and then declined above 34°C

(Trang and Giddens 1980; Sinclair and Weisz, 1985). Nodulation was ineffective in soybeans planted in very hot and dry soil environments (Greenfield, 1991).

Little information is available about the productivity of cowpea or lablab in Iowa, the need for rhizobia inoculation or fungicide seed treatment for the legume or how these crops are affected by the combination of rhizobia inoculation and fungicide seed treatments. Consequently, we conducted a field study with the objectives of determining the influence of fungicide seed treatment and inoculation on nodulation, potential for nitrogen fixation and productivity of soybean, cowpea and lablab in central Iowa.

Materials and methods

In 2013 and 2014, field experiments were conducted in Ames and Boone, Iowa respectively. In 2013, the field site and trials were established at the Iowa State University Curtiss farm (42°00'18.0"N 93°40'09.3"W). In 2014, the study was conducted at the Agricultural Engineering and Agronomy Research farm (42° 1'18.76"N 93°46'35.94"W). Both farms are drained by tiles and ditches because of poor natural drainage. Soil samples were collected before planting and samples were analyzed for pH, nitrate, available P and K (Mehlich-3), organic matter and electrical conductivity at the Soil and Plant Analysis Laboratory, Iowa State University (Table 1). The predominant soils at the Curtiss farm were Canisteo clay loam (Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) (NRCS, 2015) and Nicollet loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls) (NRCS, 2011) (Table 2). The soils at the Agricultural Engineering and Agronomy Research farm were predominantly Canisteo silty clay loam and Clarion loam (Fine-loamy, mixed, superactive, mesic Typic Hapludolls) (NRCS, 2005), Bemis moraine soils (Table 3). Both sites had been in a maize-soybean rotation

for several decades with no known production history of cowpea or lablab production in previous years.

The experimental design was a randomized complete block with a complete factorial of fungicide seed treatment, *Bradyrhizobium* inoculation, and pulse crop. Fungicide treatment, mefenoxam + fludioxonil (ApronMaxx) was applied to seed at 3.3 ml kg seed^{-1} about 2 weeks before planting. N-DURE Premium (Verdesian Life Sciences, Cary NC) liquid commercial inoculant was used in 2013 and EL type inoculant (peat-based) (INTX Microbials, LLC, Kendall, IN) was used in 2014 to inoculate seeds at labeled rates prior planting. The four pulse crops included two cowpea cultivars, California black-eyed pea, 'CA46' and purple hull pinkeye southern pea, 'Top Crop', Lablab 'Rongai' and soybean variety '92Y82' (DuPont Pioneer, Johnston, IA). Each experimental site had 4 complete blocks with each block consisting of 16 treatments. 8 were rhizobium inoculated treatments (4 had fungicide treatment and 4 only had rhizobium without fungicide) and 8 uninoculated treatments (4 with fungicide treatment and 4 without rhizobium or fungicide). Individual plot size was 3.1m wide by 7.6 m long with 30-inch row spacing. The seeding rate was 34 pure live seed (PLS) m^{-2} and plots were planted on 6 and 13, June 2014 and 2013 respectively. The seeds were planted at a depth of about 2.5cm.

Fertilizer was not applied on site at the time of the experiment because available nutrient concentrations were adequate for soybean 'P92Y82' production in Iowa (Mallarino et al., 2013). A pre-plant application of pendimethelin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) in 187 L water ha^{-1} was done 2013 and 2014. Residual weed management was done with a combination of cultivation, hand hoeing and hand pulling. In 2013, a weed infestation later in the season in soybean 'P92Y82' plots was controlled by glyphosate [N-(phosphonomethyl)glycine] at 3.36 kg a.i. ha^{-1} in 93 L H_2O. Lambda-cyhalothrin

([1α(S*),3α(Z)]-(±)-cyano-(3-phenyloxyphenyl)methyl-3-(2-chloro-3,3,3,-tricfluoro-1-propenyl)-2,2-dimethylcycloprpanecarboxylate) was used to control Japanese beetles (*Popillia japonica*) in 2013 and leafhoppers (*Empoasca fabae*) in 2013 and 2014 early in the season. The latter was applied at 9.08 g a.i. ha^{-1} in 93 L H_2O with a 0.762m open boom sprayer. The experiment relied solely on natural rainfall and plots were not irrigated and all the water was received from rainfall.

Phenological stage was determined weekly from the two middle rows of each plot using the staging system (Fehr et al., 1971). Stand counts were taken when plants were between V4 and V6 stages using a randomly placed 5.3-m measuring rod along the length of each row. At R4, four random plants were selected per plot; two plants from each of the two outer rows were dug from the ground with enough soil to avoid losing nodules. The uprooted plants were soaked in buckets of water at ambient temperature and plant roots were carefully washed to prevent nodule loss. The number of nodules per plant was counted and the taproot sliced to check for symptoms of root diseases. Ten representative nodules per plot were dissected to determine nodule color and the number of pink or red nodules recorded. Aboveground biomass was determined at R5.5, prior to leaf loss, by hand-clipping 1 m-row from each plot. Samples were placed in a forced air oven at 60^{o} C until dry, and then weighed and ground. At developmental stage R8, the pulses were hand harvested for subsequent determination of yield components. Pods were hand harvested from 2-m of row from the two central rows of each plot. Plots were harvested on 10 and 17 October in 2013 and 2014, respectively. The pods were counted as they were taken off each stem and the number of pods m^2 calculated. Pods were then threshed with a stationary thresher (Model Almaco BT-14, Nevada, IA). Seeds were counted using a Seedburo 801 Count-A-Pak (Seedburo Equipment, Des Plaines, IL) seed counter. The seeds were weighed

using a Balanza OHaus Explorer scale (Model E1B120, Parsippany, NJ) after drying in an oven over night at 60^{o}C. Crude protein, oil and fiber analyses were determined for soybean 'P92Y82' grain using Near Infrared Spectroscopy, NIRS (Kovalenko et al., 2006). A subsample of cowpea seed from each plot was ground to pass a 1.0-mm sieve with a cyclone mill and used for total N determination using colorimetric procedures (LECO, St. Joseph, MO). Crude protein was calculated as N × 6.25.

Data were analyzed by generalized linear mixed models (PROC GLIMMIX, (SAS v9.4). Blocks were random elements in the model while fungicide, *Bradyrhizobium*, and pulse crop factors were main fixed effects. The PDIFF procedure was used to test for differences among means when F-tests were significant for main effects and their interactions. Differences between treatments are reported at a significance level of 0.05.

Results

During the growing season, temperatures tended to be warmer than average at both experimental sites. Average temperatures were slightly warmer in 2013 than 2014. Additionally, average temperatures in 2013 were 1.0˚C to 4.0˚C higher compared to 2014 temperatures (Table 4). Precipitation during the growing season was less in 2013 than during the growing season in 2014. In addition, precipitation in 2013 from May to October was 34% lower than the past 12-year average whereas that in 2014 was 20% more than the past 12-year precipitation average (Table 4). From June to October, 2014 received 75% more rain than 2013 (Table 4).

The interaction of fungicide and *Bradyrhizobium* seed treatments was not significant for any of the response variables measured in this study; however, the effect of crop was significant for all response variables. Stand density varied among crops at V6 (Table 5). 'Top Crop' cowpea

had the greatest stand density, followed by ‘CA46’. soybean ‘P92Y82’ and lablab had lower stand density than ‘CA46’ and did not differ from one another. Stand density was not significantly different for seed fungicide, seed rhizobium inoculation, or interactions of seed treatments with crop (Table 5).

Plant height differed amongst crop but other main effects and all interactions were non-significant (Table 5). The cowpea ‘CA46’ was the tallest and ‘Top Crop’ was the shortest at crop maturity. ‘CA46’ was 28% and 62% taller than soybean ‘P92Y82’ and ‘Top Crop’ at R8. Since lablab did not reach maturity, plant height was not taken at R8 for the crop.

The number of nodules per plant was significantly different among crops. Soybean ‘P92Y82’ had the greatest number of nodules followed by ‘CA46’ and ‘Top Crop’; lablab had the least number of nodules. Although lablab had the least number of nodules, lablab nodules were observed to be larger in size compared to soybean ‘P92Y82’, ‘CA46’ and ‘Top Crop’. Fungicide or *Bradyrhizobium* application to seeds did not significantly affect the total number of nodules per plant. All crops that received both fungicide and *Bradyrhizobium* had statistically similar nodule numbers to their uninoculated controls.

The number of red nodules per plant differed among the four pulse crops, but the effects of fungicide and *Bradyrhizobium* application and all interactions were nonsignificant (Table 5). P92Y82 had the greatest number of red nodules per plant followed by ‘CA46’ and then ‘Top Crop’. Lablab had the fewest number of red nodules per plant.

Above ground biomass differed significantly by crop although seed application of fungicide or *Bradyrhizobium*, and all interactions, had no significant impact (Table 5). Soybean ‘P92Y82’ had the greatest amount of aboveground biomass followed by ‘CA46’, lablab and ‘Top Crop’ had the lowest. Soybean ‘P92Y82’ had 18%, 25% and 29% more aboveground biomass

than ‘CA46’, lablab and ‘Top Crop’ respectively. The impact of fungicide and *Bradyrhizobia* application on aboveground biomass harvested at R 7.5 was not significant.

Seed yield differed significantly for crop but the effects of seed fungicide and *Bradyrhizobium* and interactions were not significant (Table 5). Soybean ‘P92Y82’ had greater yield compared to ‘Top Crop’ and ‘CA46’, 49 and 55% greater yield than these cowpeas, respectively. The two cowpeas had similar yields. Lablab did not reach physiological maturity in either 2013 or 2014, remaining vegetative until killing frost occurred. Fungicide and rhizobium treatment had no significant impact on yield.

Pod density was significantly different among crops (Table 5). Soybean ‘P92Y82’ had more pods m^{-2} than ‘Top Crop’ and ‘CA46’. ‘Top Crop’ had 21% more pods than ‘CA46’. S soybean ‘P92Y82’ had 77 and 82% greater pod density than ‘Top Crop’ and ‘CA46’ respectively. The influence of seed fungicide, rhizobium inoculation, and their interaction was not significant on pod density (Table 5). The number of seed per pod differed significantly by crop but effects of but fungicide and *Bradyrhizobium* seed treatments and interactions were not significant (Table 5). ‘Top Crop’ and ‘CA46’ had similar numbers of seed per pod, significantly greater than that of soybean ‘P92Y82’. ‘Top Crop’ had 60% more than soybean ‘P92Y82’. The main effects of fungicide and *Bradyrhizobium* application and interactions did not influence seed per pod. Individual seed weight was significantly different among crops (Table 5). Soybean ‘P92Y82’ had the greatest seed weight and ‘Top Crop’ seeds weighed the least while seed of ‘CA46’ were intermediate and different from other crops. Soybean ‘P92Y82’ seeds weighed 6.4 and 12% more than ‘CA46’. Fungicide and *Bradyrhizobium* treatment had no significant impact on individual seed weight and therefore weight of seeds from plots that received fungicide and *Bradyrhizobia* treatments was not statistically different from seed weight from control plots.

Seed number m^{-2} differed by crop (Table 5). Soybean 'P92Y82' produced more seed per area while 'CA46' produced the least. Soybean 'P92Y82' plots had 54 and 43% more seeds than 'CA46' and 'Top Crop', respectively. The number of seed per square meter was not influenced by fungicide or *Bradyrhizobia* application or their interaction.

Seed crude protein was significantly different amongst crops (Table 5). Soybean 'P92Y82' seeds had the highest amount of crude protein and 'CA46' had the lowest. Soybean 'P92Y82' seeds had 35 and 28% more crude protein than 'Top Crop' and 'CA46', respectively. Fungicide and *Bradyrhizobia* treatment did not impact seed crude protein.

Discussion

The absence of significant differences in nodulation following seed application of fungicide and *Bradyrhizobium* inoculum may be attributed to several factors such as climate, soil environment and the existence of indigenous bacterial strains in the soil (Pedersen, 2003). Both of the experimental sites had multiple decades of soybean production and therefore competitive introduced *Bradyrhizobium japonicum* strains were likely present. Many soils usually harbor native rhizobium strains of different competitiveness and compatibility even before inoculation (Daramola et al., 1994). The observed differences in nodule number by species may be attributed to the host plant as well as rhizobium strain. Amarger and Lobreau (1982) reported that the number of nodules formed varied by strain in a study on faba bean (*Vicia faba* L.). Bhuvaneswari et al. (1988) reported that the efficiency of nodule initiation and development following inoculation was greatly influenced by the host species. Similar to findings from our work, they showed that *Bradyrhizobium japonicum* formed nodules with cowpea and soybean. Somasegaran and Hoben (1985) also explained that differences in nodule number by crop may be attributed to

variances in the forms of bacteroids found in the nodules of different legumes. The authors also explained that the plant largely determined the size, shape and number of these bacteroids in each nodule. This could further explain why soybean had more nodules than the other three pulse legumes whereas lablab had the least but largest nodules compared to soybean 'P92Y82', 'CA46' and 'Top Crop'. It should be noted that nodule number is a less reliable indicator of strain effectiveness hence the number of red nodules was used to determine potential for nitrogen fixation. Since the number of red nodules per plant was not significantly affected by fungicide and *Bradyrhizobium* seed treatments, this implies that plots which did or did not receive these treatments had the same level of nitrogen fixation. The reddish or pinkish color of sliced nodules indicates that nitrogen fixation was ongoing (Flynn and Idowu, 2015). This red or pink color is caused by leghemoglobin which controls the flow of oxygen to the nitrogen fixing bacteria in the root nodules.

Although fungicide and *Bradyrhizobium* treatments had no significant impact on the number of red nodules and therefore potential for nitrogen fixation in our study in Ames, Iowa, these treatments remain of great importance in aiding legume nitrogen fixation in, regions like Africa or in areas where legumes are being grown for the first time or, in soils without sufficient amounts of indigenous rhizobium strains. It is also important to use the right rhizobium strain for particular hosts or crops. Fungicide and *Bradyrhizobium* treatment had no significant impact on aboveground biomass although this response variable differed significantly by crop. Soybean 'P92Y82' had the greatest amount of above ground biomass whereas cowpea aboveground biomass was 4550 and 3958 $kgha^{-1}$. This corresponds with Rao and Northup (2009) who reported that soybean produced more biomass than cowpea for three years in a four year experimental study. The amount cowpea biomass also corresponds with previous studies by

Agza et al. (2012) who reported dry matter accumulation yields of different cowpea genotypes ranging between 2330 $kgha^{-1}$ to 7670 $kgha^{-1}$. However, the average aboveground biomass obtained from our study for 'CA46' and 'Top Crop' are lower than for Rao and Shahid (2011) who found average aboveground biomass for different cowpea genotypes to be 18100 kg ha^{-1}. Muchow (1985) further explained the differences in biomass among different crops. He reported that biomass production was a function of incident solar radiation, the proportion intercepted, the mode of interception and the respiratory as well as photosynthetic characteristics of the crop. He added that proportion of radiation intercepted is determined by the size and arrangement of leaf canopy whereas the mode of interception is a factor determined by leaf orientation within the canopy. These factors differ among soybean 'P92Y82', lablab, 'CA46' and 'Top Crop' and may have likely caused the differences in aboveground biomass by crop. Further, Somasegaran and Hoben (1985) also reported that above ground biomass accumulation is a criterion that is generally accepted for nitrogen-fixing effectiveness although nodule dry weight is another that may be employed. This corresponds to our study findings for soybean 'P92Y82' and 'CA46' aboveground biomass accumulation and their corresponding number of red nodules per plant. Yield data was collected for only three pulse legumes (soybean 'P92Y82', 'CA46' and 'Top Crop') and none on lablab. Lablab did not reach physiological maturity and therefore no yield data were collected. Lablab 'Rongai' is a short-day plant that is quite sensitive to day length and flowers best with less than 11 hours of day light although it requires ample sunlight. Day length in Ames, Iowa was between 16 to 12 hours 2013 and 2014 growing season. Lablab 'Rongai' usually seeds late and has low frost tolerance (FAO, 2015). The absence of significant differences in nodulation (number of nodules) with fungicide and *Bradyrhizobium* seed treatment in our study is similar to findings by Rajender et al. (1986) who reported no significant effect of

fungicide on nodule number in cowpea. Researchers (Andres et al., 1998; Campo and Hungria, 2000), report reduction in nodulation and yield following fungicide and *Bradyrhizobium* inoculation in legumes like soybean, we found no significant impact of seed fungicide or *Bradyrhizobium* inoculation on yield. This corresponds with Schulz et al. (2005) who reported that Metalaxyl-M + fludioxonil (ApronMaxx) did not affect the overall yield of inoculated soybean.

In 2014, yield differed significantly by crop. Soybean 'P92Y82' had greater seed yield than the two cowpeas, due to soybean 'P92Y82' having more pods per square meter and higher seed weight than 'CA46' and 'Top Crop'. Deshmukh et al. (2011) explained that in cowpeas, the overall yield depended upon the number of pods and size of the seed. These two factors are genetically determined (Willmer and Johnston, 1976; Atkins et al., 1979). soybean 'P92Y82'may also simply have higher yield potential than cowpeas in this environment due to numerous research and extension in Iowa unlike cowpeas. In addition, Bhuvaneswari et al. (1988), reported that cowpeas had a higher threshold of response to signal substances from the bacteria for nitrogen fixation than soybeans. This in turn could affect their yield compared to soybeans. In addition, environmental factors such as temperatures significantly influence flower and pod shedding in both cowpeas and soybean (Warrag and Hall, 1984;Van Schaik et al., 1958; Gibson and Mullen, 1996) and with consequent effects on yield. Although soybean 'P92Y82' yielded more than the two cowpeas, 'CA46' and 'Top Crop' had more seeds per pod than soybean 'P92Y82'. These differences in seed weight may be attributed to differences in genetic makeup of the crops (Sene, 1986; Aryeetey and Lang, 1973; Drabo et al., 1973). Seed weight is also a function of varieties used. Soybean 'P92Y82'seeds contained more crude protein than both cowpeas. This corresponds with reports by Awolumatea (1983) who found that, although

cowpeas accumulated nitrogen at a rate much faster than soybean during seed development, 994 μg day^{-1} compared to 473 μg day^{-1} respectively, crude protein of biomass decreased with development time from 40% in early seed development stages to 26% in mature cowpea and from 35 to 33% in soybeans. The greater decrease in cowpea crude protein at maturity compared to soybean could explain why soybean 'P92Y82' seeds contained more crude protein than cowpeas.

Conclusions

The application of fungicide and *Bradyrhizobium* seed treatments in this study did not significantly affect the total number of nodules, red nodules yield or aboveground biomass. However, yield, above ground biomass, total number of nodules, red nodules, and yield components differed among pulse crops. Cowpea may not require rhizobium inoculation to obtain N fixation in Iowa but it is likely that lablab would require inoculation. The abundance of more crude protein in soybean 'P92Y82' seeds at maturity compared higher levels of crude protein in cowpea during early reproductive development may be important in deciding at what stage to best utilize either cowpea or soybean in order to utilize N as cover crop or leafy vegetable for human consumption. These differences in apparent N translocation also require additional research in order to be better understood. Overall, soybean 'P92Y82' performed better than cowpeas 'CA46' and 'Top Crop', and lablab for nodulation, aboveground biomass accumulation, yield and seed crude protein which may be because soybean 'P92Y82' is better adapted to the climate and environment of Iowa due to long term efforts by plant breeders working on soybean improvement.

Acknowledgements

The authors are grateful for technical assistance from Gary Hammitt, Danielle Wilson, Luke Hodnefield and Roger Hintz and the help of several graduate and undergraduate students in the field and the laboratory.

Table 1. Pre-plant soil test values for Story County (2013) and Boone (2014) experimental Sites.

Year	Depth	P mg kg^{-1}	K mg kg^{-1}	pH	OM g kg^{-1}	NO_3 mg kg^{-1}	EC dS m^{-1}
2013	0 - 15 cm	35	198	5.7	46	4	1.152
2013	15 - 30 cm	7	136	6.5	42	5	0.35
2014	0 - 15 cm	43	153	5.5	36	7	-
2014	15 - 30cm	14	99	5.6	27	4	-

Table 2. Predominant soils, Curtiss farm, Ames, Iowa (2013).

Story County, Iowa (IA169)		
Predominant soils	Acres in AOI	Percent of AOI
Canisteo clay loam, 0 to 2 percent slopes	0.2	67.40%
Nicollet loam, 1 to 3 percent slopes	0.1	32.60%
Totals for AOI	0.3	100.00%

AOI means Area of Interest

Table 3. Predominant soils, Agricultural Engineering and Agronomy Research farm, Boone, Iowa (2014).

Boone County, Iowa (IA015)		
Predominant soils	Acres in AOI	Percent of AOI
Canisteo silty clay loam, 0 to 2 percent slopes	5.3	60.20%
Clarion loam, Bemis moraine, 2 to 6 percent slopes	3.5	39.80%
Totals for AOI	8.9	100.00%

AOI means Area of Interest

Table 4. Long-term monthly average air temperature and total precipitation during the two-year study.

Month	2014	2013	Long-term (12 year total average)
	Monthly mean air temperature (ºC)		
April	10	8	11
May	17	16	17
June	22	21	22
July	22	23	24
August	22	23	23
September	17	21	18
October	11	11	11
	Total precipitation (mm)		
April	121	148	98
May	108	180	136
June	225	26	122
July	73	26	115
August	148	30	130
September	138	30	85
October	119	64	65

Table 5. Stand density, height, nodule number, biomass, seed yield, yield components, and seed crude protein for four pulse crops for 2013 and 2014 in Central Iowa.

	Stand V6 no. m^{-2}	Height R8 Cm	Total nodule no. $plant^{-1}$	Red nodule no. $plant^{-1}$	Biomass kg ha^{-1}	Yield kg ha^{-1}	Pods no. m^{-2}	Seed no. pod^{-1}	Seed mg $seed^{-1}$	Seed no. m^{-2}	Seed CP g kg^{-1}
Fungicide											
Applied	24.5	110	21.7	17.6	4618	2061	800	4.2	163	2440	274
Not applied	25.0	100	21.5	16.9	4463	2031	793	4.2	160	2455	279
Rhizobium											
Applied	24.8	101	21.7	17.3	4548	2021	784	4.1	162	2391	275
Not applied	25.0	109	21.6	17.2	4534	2070	809	4.3	161	2503	277
Crop											
Soybean	21.0 c	108 b	37.2 a	28.5 a	5543 a	3130 a	1692 a	2.1 b	172 a	3614 a	350 a
Lablab	21.5 c	-	2.5 c	2.5 d	4112 bc	-	-	-	-	-	-
CA46	26.2 b	151 a	25.9 b	23.2 b	4550 b	1408 b	309 c	5.2 a	161 b	1651 c	226 c
Top Crop	29.8 a	57 c	21.0 b	14.8 c	3958 c	1602 b	390 b	5.3 a	152 c	2077 b	253 b
Significance						*P* > F					
Fungicide (F)	ns	ns	ns	Ns	ns	ns	ns	ns	ns	Ns	ns
Rhizobium (R)	ns	ns	ns	Ns	ns	ns	ns	ns	ns	Ns	ns
F × R	ns	ns	ns	Ns	ns	ns	ns	ns	ns	Ns	ns
Crop (C)	***	***	***	***	***	***	***	***	***	***	***
F × C	ns	ns	ns	Ns	ns	ns	ns	ns	ns	Ns	ns
R × C	ns	ns	ns	Ns	ns	ns	ns	ns	ns	Ns	ns
F × R × C	ns	ns	ns	Ns	ns	ns	ns	ns	ns	Ns	ns

***Significant at = 0.001

V6 -Vegetative stage 6

R8 – Reproductive stage 8

REFERENCES

Agza, B., K. Binyam, Z. Solomon, A. Eskinde and A. Ferede. 2012. Animal feed potential and adaptability of some cowpea (*Vigna unguiculata*) varieties in northwest lowlands of Ethiopia. J. Agric. Res. 11:478 – 483.

Allen, D.J. and O.T. Edje. 1990. Common bean in Africa farming systems. Progress in the improvement of common bean in Eastern and Southern Africa. African workshop series.

Amarger, N., and J.P. Lobreau. 1982. Quntitative study of nodulation competitiveness in Rhizobium strains. Appl. Environ. Microb. 44:583–588.

Awolumatea, E. 1983. Accumulation and quality of storage protein in developing cowpea , mung bean and soya bean seeds. J. Sci. Food. Agri. 34:1351–1357.

Badi, S.H., H.D. Dikwahal, and G.G. Jibung. 2012. Response of vegetable cowpea (*Vigna unguiculata*) to intra-row spacing and defoliation at Garkawa. Asian J. Agric. Sci. 4:210–212.

Baloyi, B.M., and V.I. Ayodele. 2013. Effects of leaf harvest on crude protein and mineral contents of selected early maturing lines of lablab (*Lablab purpureus*). Afr. J. Agric. Res. 8:449–453.

Bayne, H.G., M.S. Brown, and G.J. Bethlenfalvay. 1984. Defoliation effects on mycorrhizal colonization , nitrogen fixation and photosynthesis in the *Glyicne-Glomus-Rhizobium* symbiosis. Physiol. Plant. 62:576–580.

Bhuvaneswari, T.V, A.P. Lesniak, and W.D. Bauer. 1988. Efficiency of nodule initiation in cowpea and soybean. Plant. Physiol. 86:1210–1215.

Bikrol, A., N. Saxena, and K. Singh. 2005. Response of *Glycine max* in relation to nitrogen fixation as influenced by fungicide seed treatment. Afr. J. Biotechnol. 4:667–671.

Blount, A.R.S., D.L. Wright, R.K. Sprenkel, T.D. Hewitt, and R.O. Myer. 2009. Forage soybeans for grazing , hay and silage. University of Florida IFAS Extension. Publication #SS-AGR-1801,1-5. https://edis.ifas.ufl.edu/ag184 (accessed 30 Nov. 2015).

Board, J.E., A.T. Wier, and D.J. Boethel. 1994. Soybean yield reductions caused by defoliation during mid to late seed filling. Agron. J. 86:1074–1079.

Brkić, S., Z. Milaković, A. Kristek, and M. Antunović. 2004. Pea yield and its quality depending on inoculation , nitrogen and molybdenum fertilization. Plant Soil. Environ. 50:39–45.

Browde, J.A., L.E. Pedigo, M.D.K. Owen, G.L. Tylka, and B.C. Levene. 1994. Growth of soybean stressed by nematodes , herbicides , and simulated insect defoliation. Agron. J. 86:968–974.

Burton, J.W., D.W. Israel, R.F. Wilson, and T.E. Carter. 1994. Effects of defoliation on seed protein concentration in normal and high protein lines of soybean. Plant. Soil. 172:131–139.

Corriher-olson, V., and G.R. Smith. 2013. Establishment of tropical annual legumes sod-seeded into bermudagrass or prepared seedbed. Tex. J. Agri. Nat. Resourc. 26:73–81.

Demooy, B.E., and C.J. Demooy. 1989. Effects of leaf-harvesting practices on yield and yield components of ER-7 cowpea (*Vigna unguiculata*) in semi-arid Botswana. Field. Crops. Res. 22: 27–31.

Deshmukh, D.V, S.N. Mate, R.W. Bharud, and P.N. Harer. 2011. Analysis of pod and seed development in cowpea [*Vigna unguiculata* (L .) Walp]. Am. Eurasian. J. Agron. 4:50–56.

Drabo, I., R. Redden, J.B. Smithson, and V.D. Aggarwal. 1973. Inheritance of seed size in cowpea [*Vigna unguiculata* (L .) Walp.]. Euphytica. 33: 929–934.

Ehlers, J.D., and A.E. Hall. 1997. Cowpea [*Vigna unguiculata* (L.) Walp.]. Field Crops Res. 53:187–204.

Fening, J., and S.K. Danso. 2002. Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. Appl. Soil. Ecol. 21:23–29.

Figueiredo, M.V.B., J.J. Vilar, H.A. Burity, and F.P. de França. 1999. Alleviation of water stress effects in cowpea by *Bradyrhizobium* spp . inoculation. Plant. Soil. 207:67–75.

Flynn, R. and J. Idowu. 2015. Nitrogen fixation by legumes. http://aces.nmsu.edu/pubs/_a/A129/ (Accessed 30 Nov. 2015).

Gazzoni, C.I.O.L., and V.I.O. Moscardi. 1997. Effect of defoliation levels on recovery of leaf area , on yield and agronomic traits of soybeans. Braz. J. Agric. Res. 33:411-424.

Gibson, L.R., and R.E. Mullen. 1996. Influence of day and night temperature on soybean seed yield. Crop. Sci. 36:98–104.

Grymes, C.F., J.L. Griffin, D.J. Boethel, B.R. Leonard, L. David, J. S. Russin, and D. L. Jordan. 1999. Soybean response to weed interference and defoliation. Weed Sci. 47:90–94.

Hammond, R.B. 1988. Effects of leaf removal at soybean growth stage V1 on yield and other growth parameters. J. Kans. Entomol. Soc. 62:96–102.

Harrison, H.F., J.A. Thies, R.L. Fery, and J.P. Smith. 2006. Evaluation of cowpea genotypes for use as a cover crop. Hortsci. 41:1145–1148.

Hashem, F.M., S.A. Saleh, P.V. Berkum, and M. Voll. 1997. Survival of *Bradyrhizobium* sp. (*Arachis hypogaea* L.) on fungicide-treated peanut seed in relationship to plant growth and yield. World J. Microbiol. Biotechnol. 13:335–340.

Hikosaka, K., T. Takashima, D. Kabeya, T. Hirose, and N. Kamata. 2005. Biomass allocation and leaf chemical defence in defoliated seedlings of *Quercus serrata* with respect to carbon-nitrogen balance. Ann. Bot. 95:1025–1032.

Hintz, R.W., H.H. Beeghly, W.R. Fehr, A.A. Schneiter, and D.R. Hicks. 1991.Soybean response to stem cutoff and defoliation during vegetative development. J. Prod. Agric. 4:585–589.

Hirsch, P.R. 1996. Population dynamics of indigenous and genetically modified rhizobia in the field. New Phytol. 133:159–171.

Hofstrand, J., 2010. Economics of tile drainage. https://www.extension.iastate.edu/agdm/articles/hof/HofJuly10.html (Accessed 1 Dec 2015).

Johnston, T.J., and J.W. Pendleton. 1968. Contribution of leaves at different canopy levels to seed production of upright and lodged soybeans (*Glycine max* (L.) Merrill)1. Crop. Sci. 8:291–292.

Kovalenko, I.V., G.R. Rippke, and C.R. Hurburgh. 2006. Determination of amino acid composition of soybeans (*Glycine max*) by Near-Infrared Spectroscopy. J. Agric. Food Chem. 54: 3485-3491.

Klubertanz, T. H., L.P. Pedigo, and R.E. Carlson. 1996. Soybean physiology, regrowth, and senescence in response to defoliation. Agron. J. 88:577–582.

Kutcher, H.R., G. Lafond, A.M. Johnston, P.R. Miller, K.S. Gill, W.E. May. 2002. Rhizobium inoculant and seed-applied fungicide effects on field pea production. Can. J. Plant. Sci. 82: 645–661.

Law, I.J., W.F. Botha, U.C. Majaule, and F.L. Phalane. 2006. Symbiotic and genomic diversity of “cowpea” bradyrhizobia from soils in Botswana and South Africa. Biol. Fertil. Soils. 43:653–663.

Lovvorn, R.L., and B.W. Smith. 1943. Response of soybeans to experimental defoliation. J. Am. Soc. Agron. 35:768–778.

Maass, B.L., M.R. Knox, S.C. Venkatesha, T.T. Angessa, S. Ramme and B.C. Pengelly. 2010. *Lablab purpureus*-A Crop Lost for Africa? Trop. Plant. Biol. 3:123–135.

Martins, L.M.V., G.R. Xavier, F.W. Rangel, J.R.A. Ribeiro, M.C.P. Neves, L.B. Morgado, and N.G. Rumjanek. 2003. Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. Biol. Fertil. Soils. 38:333–339.

Mcalister, D.F., and O.A. Krober. 1958. Response of soybeans to leaf and pod removal. Agron. J. 50:674–677.

Midega, C.A.O., D. Salifu, T.J. Bruce, J. Pittchar, J.A. Pickett, and Z.R. Khan. 2014. Cumulative effects and economic benefits of intercropping maize with food legumes on *Striga hermonthica* infestation. Field Crops. Res. 155:144–152.

Muthomi, J.W., P.E. Otieno, G.N. Chemining'wa, and J.H. Nderitu. 2007. Effect of root rot pathogens and fungicide seed treatment on nodulation in food grain legumes. J. Biol. Sci. 7:1163–1170.

Ogedegbe S.A, V.B. Ongunlela, O.O. Olufajo, and E.C. Odion, 2012. Seed yield and yield attributes of lablab as influenced by phosphorous application, cutting height. Asian. J. Crop. Sci. 4:12–22.

Ohler, T.A, S.S. Nielsen, and C.A. Mitchell. 1996. Varying plant density and harvest time to optimize cowpea leaf yield and nutrient content. Am. Soc. Hortic. Sci. 31:193–197.

Oelberg, K. 1956. Factors affecting the nutritive value of range forage. J. Range. Manage. 9: 220–225.

Otieno, P.E., J.W. Muthomi, G.N. Chemining'wa, and J.H. Nderitu. 2007. Effect of rhizobia inoculation, farmyard manure and nitrogen fertilizer on growth, nodulation and yield of selected food grain legumes. Afr. Crop. Sci. Soc. 8:305–312.

Pickle, C.S., and C.E. Caviness. 1983.Yield reduction from defoliation and plant cutoff of determinate and semideterminate soybean. Agron. J. 4:474–476.

Proulx, R.A., and S.L. Naeve. 2009. Pod removal, shade, and defoliation effects on soybean yield, protein, and oil. Agron. J. 101:971–978.

Rao, S.C., and B.K. Northup. 2009. Capabilities of four novel warm-season legumes in the southern great plains: Biomass and forage quality. Crop. Sci. 49:1096–1102.

Rennie, R.J., and S. Dubetz. 1982. Effect of fungicides and herbicides on nodulation and N_2 fixation in soybean fields lacking indigenous *Rhizobium japonicum*. Agron. J. 76:451–454.

Saidi, M., M. Ngouajio, F.M. Itulya, and J. Ehlers. 2007. Leaf harvesting initiation time and frequency aff ect biomass partitioning and yield of cowpea. Crop. Sci. 47:1159–1166.

Schulz, T.J., and K.D. Thelen. 2008. Soybean seed inoculant and fungicidal seed treatment effects on soybean. Crop. Sci. 48:1975–1983.

Schulz, T.J., K.D.Thelen, and D. Wang. 2005. The effect of *Bradyrhizobium japonicum* inoculant on soybean growth and yield. http://www.researchgate.net/publication/237651647_The_Effect_of_*Bradyrhizobium_japonicum*_inoculant_on_soybean_growth_and_yield (Accessed 30 Nov. 2015).

Somasegaran, P., and H.J. Hoben. 1985. Methods in legume-*Rhizobium* technology. University of Hawaii NifTAL. Hawaii Institute of tropical agriculture and human resources.

Teigen, J.B., and J.J. Vorst. 1975. Soybean response to stand reduction and defoliation. Agron. J. 67:813–816.

Van Kessel, C., and C. Hartley. 2000. Agricultural managemnet of grain legumes: has it led to an increase in nitrogen fixation? Field Crops Res. 65, 165-181.

Wood,I. 1983. Lablab bean (*Lablab purpureus*) for grain and forage production in the Ord irrigation area. Aust. J. Exp. Agric. 23:121-162.

CHAPTER 3

NUTRITIONAL COMPOSITION OF PULSE LEGUME LEAVES AND THE IMPACT OF LEAF REMOVAL ON YIELD

A manuscript for submission to Field Crops Research

Abstract

The role of legumes worldwide includes food, nutrition and income generation among others. Malnutrition especially in children is common in many developing countries, leading to severe stunting and death in addition to high macro and micro nutrient deficiencies which legumes can provide. Legumes such as cowpea can thrive in adverse environments like drought, making them a climate smart technology for hunger mitigation. Total protein intake per capita from pulses exceeds 10% in several countries, including Uganda (20%) and Ethiopia (15%), yet potential legume leaf utilization for protein and other nutrients has not been widely considered. This may be a rare source of nutritional and harvest versatility compared to common leafy vegetables. However, leaf removal effects on pulses and their grain yield are unclear. Additionally, little information is available on pulse leaf nutritional composition. We conducted a two-year experiment in central Iowa to determine 1) the effects of leaf removal rate on nutritive value of removed leaf tissue, and 2) the effects of leaf removal rate on subsequent grain yield. We found that mean concentration of nutrients in dry leaves was 229, 17832, 4461, 21991, 3702, 113, 205, 13, 86, 2806 mg kg^{-1} for crude protein, calcium, Mg, K, P, Mn, Fe, Cu, Zn and S. Unlike 2013, leaf removal percentage had significant impact on 2014 yield and the control had 20, 32, and 35% more yield than plots at 33, 66 and 99% leaf removal. Pulse leaves have

excellent potential to supplement high-carbohydrate diets that are deficient in protein and micronutrients.

Keywords: Cowpea; California Black-eyed pea, 'CA46'; 'Top Crop'; 'Iron and Clay'; Lablab; Soybean; Leaf removal, Nutrition, Yield

Introduction

The increasing need for protein to meet human nutritional needs and livestock industry worldwide necessitate a cheap source of both leafy vegetables and forage. For the livestock industry, supplementary pastures are essential for economical production in both the beef and dairy cattle (*Bos taurus* L.) sectors, especially in regions with inadequate permanent pastures or dry seasons with poor forage productivity (Lovvorn and Smith, 1943). In Asia and Africa, consumption of young, tender leaves by humans and utilization of older leaves as forages is common for legumes such as lablab [*Lablab purpureus* (L.) Sweet], also known as hyacinth bean, and cowpea [*Vigna unguiculata* (L.) Walp.] (Saidi et al., 2007; Baloyi and Ayodele, 2013). Cowpea in particular is among the top three or four leafy vegetables in many African countries (Barrett, 1990). In Nigeria and Botswana, for instance, prostrate varieties of legumes like cowpeas with long vines are utilized for their leaves and young green pods (Badi et al., 2012). The consumption of legume products has also proven to reduce the risk of a number of chronic diseases (Gundgaard et al., 2003; Gossalau and Chen, 2004). In Botswana, vegetable green leaves are harvested throughout the growing season at all stages of development though the effect of leaf harvesting on yield is not well documented (Demooy and Demooy, 1989). Farmers still lack sufficient knowledge about the number of leaves to be harvested and at what

interval these leaves may be harvested without affecting green pod or seed yield at harvest (Badi et al., 2012).

The consumption of legume leaves provides nutrients to humans and animals and legumes such as cowpea and lablab are documented to thrive in regions with drought and low soil fertility. Lablab yields greater aboveground biomass under drought conditions compared to cowpea and soybean [*Glycine max* (L.) Merr.] (Murphy and Colucci, 1999). Even with these advantages, it is not well documented how lablab, cowpea or soybean are affected by leaf removal. Crawley (1983) reported that growth and reproduction significantly decreased when plants were subjected to herbivory or defoliation. Herbivory also resulted in alteration of the chemical composition of the remaining or newly flushing leaves such as in solanaceous plants (Karban and Myers, 1989; Karban and Baldwin, 1997). Although legumes such as soybeans are known to have the potential to recover from physical damage, especially if such damage is minimal and inflicted during early stages of plant growth (Teigen and Vorst, 1975), timing of defoliation is important and may influence grain yield.

Fehr et al. (1981) and Board et al. (1984) demonstrated that stages R5 or R5.5 were the most sensitive to defoliation in both determinate and indeterminate soybean cultivars leading to 80% yield reduction with 100% defoliation. McAlister and Krober (1958) reported that 40% defoliation at a growth stage close to seed initiation in soybean led to a 9% reduction in yield and 80% defoliation led to yield reduction of up to 32%. Adverse effects of defoliation were observed when leaf harvesting was done during early podding stage for cowpeas, soybeans, groundnut (*Arachis hypogaea* L.) and green gram [*Vigna radiata* (L.) R. Wilczek] (Enyi, 1975). Greatest yield reduction in soybeans was observed when defoliation was done at the beginning of seed formation whereas the least reduction in yield was associated with defoliation done just

before flowering (Camery and Weber, 1953). However, some studies report that defoliation increased dry matter accumulation per plant by decreasing competition for light and therefore enabling light to reach lower leaves which increased photosynthetic rate per plant in soybean (Teigen and Vorst, 1975). Defoliation also increased the efficiency of remaining leaves which compensated for the leaves removed (Monteith, 1977). The increased efficiency of remaining minimally damaged leaves reduced yield very little at 50% defoliation compared to the control in soybean when it was done between appearance of the first trifoliate leaf and full flowering (Weber, 1955). Weber (1955) reported that their control had 5% more yield with 50% defoliation and 0% topping at V5. In other studies, defoliation during vegetative stages had minimal impact on yield because of potential for leaf regrowth (Board et al., 1984).

The intensity of defoliation may affect yield and a number of studies reported yield reduction with various defoliation levels. With 100% defoliation, soybean yield declined by 20% (Weber, 1955). Studies by Goli and Weaver (1986) also reported greater reduction in yield when 100% of the leaves were harvested at R4 or R5 compared to R6. Yield reduced by 13 to 17% with 50% defoliation during R4 to R5 stages (Caviness and Thomas, 1980). Board et al. (1984) explained that as the seed filling period (R5-R7) progresses, yield sensitivity to defoliation increases. Other authors added that plant defoliation affected yield differently, for instance, number of seed and pods was reduced with defoliation (McAlister and Krober, 1958; Caviness and Thomas, 1980; Board and Harville, 1993), whereas defoliation affected seed size of soybean (Egli and Leggett, 1976; Fehr et al., 1981; Ingram et al., 1981). These differences in yield effects were also attributed to the differences in the stages at which defoliation was done (Board et al., 1984). Board and Harville (1993) reported that defoliation during early reproductive stages of soybean primarily affected pod number. As the plant responds to a lower Leaf Area Index (LAI)

and light interception, pod number was reduced but seeds per pod or seed weight remain unaffected during seed filling (Board et al., 1984). However, individual seed weight decreased when defoliation occurred during the seed filling period (Ingram et al., 1981; Goli and Weaver, 1986). Gbikpi and Crookston (1981) explained that individual seed weight was determined by the seed filling rate and the effective filling period. Yield was influenced by the seed filling rate during the effective filling period (Kaplan and Kooler, 1974; Egli et al., 1984).

Klubertanz et al. (1996) documented that defoliated stressed soybeans conserved more water than non-defoliated stressed soybean plants and also reported that senescence of lower leaves was delayed in defoliated plants. However, the effect of defoliation on the physiology of remaining leaves varied from one plant species to another (Welter, 1989). Leaf area compensation following defoliation was expressed through changes in new leaf area expansion or in terms of normal plant senescence in soybeans (Gazzoni and Moscardi, 1997). Gazzoni (1974) reported that high recovery was observed when defoliation was done at vegetative stages and even higher recovery indexes where higher defoliation levels were applied. Gazzoni and Moscardi (1997) reported that low and medium defoliation done at reproductive stages led to reduction of leaf area beyond natural senescence whereas total plant defoliation induced a minimal/ less recovery of the leaf area in soybean. However, Fehr et al. (1981) demonstrated that development of new leaf area after defoliation at R4 and R4.5 was higher for an indeterminate variety and negligible for defoliations done at R5.5 and R6. They further reported that differences in yield decrease may not be due to differences in leaf area recovery. Although Board et al. (1994) attributed the minimal yield effect following defoliation at vegetative stages to the potential for leaf regrowth during those stages of growth. Higgins et al. (1983) also reported no compensatory regrowth in leaf area of soybean following defoliation. Compensatory

regrowth was minimal because defoliated soybean plants had greater leaf area in the lower abscission stratum and minimal evidence of compensation on the higher stratum except in one year of three-year studies were conducted (Ostlie and Pedigo, 1985).

Leaf removal may also affect subsequent dry matter accumulation. Defoliation accelerated shoot growth at the expense of root growth in herbaceous plants (Wilson, 1988). On the other hand, the shoot to root ratio following defoliation recovered to a value similar to a value before leaf removal in cowpea and common bean respectively (Huxley and Sammerfield, 1976; Fondy and Geiger, 1980). Gazzoni and Moscardi (1997) reported that the rate of soybean dry matter accumulation in both vegetative tissue and total plant decreased due to reduced growth and leaf fall. They explained that the decrease in leaf and stem nitrogen content after bloom was an indicator of translocation to pods and seeds in spite of total nitrogen accumulation continuing late in the growth cycle. Phosphorous and potassium reached a peak during pod filling stage and it is then that the translocation of pods and seeds triggered reduction of their (potassium and phosphorous) content in the vegetative parts of the plant (Gazzoni and Moscardi, 1997). This accounts for yield reductions following medium to high defoliation done between pod fill and physiological maturity (Gazzoni and Moscardi, 1997). The authors further explained that this may also explain the low impact of defoliation when done at vegetative stages since plants have the potential as well as time to regenerate loss of photosynthates that were stored in the harvested or removed leaves.

Defoliation/leaf removal may affect seed nutritional composition. Soybean seed protein was reduced by 1% following 100% plant defoliation (Camery and Weber, 1953). Soybean seed oil, protein, seed weight and yield also decreased with 80% defoliation although the iodine in seed oil increased (McAlister and Krober, 1958). Proulx and Naeve (2009) reported that yield

formation in grain crops involved the production of carbohydrate, protein and oil by actively growing seeds. The authors added that this process relied on the availability of raw materials supplied by the plant in the form of C and N assimilates. Whereas most of the C is abundant in phloem as sucrose in soybean (Layzell and LaRue, 1982; Rainbird et al., 1984), nitrogen is acquired by uptake and reduction of soil nitrate as well as fixation by *Bradyrhizobium* in root nodules (Harper, 1987). Carbon supply to seeds was observed to decline beginning at the onset of senescence (Buchanan-Wollaston, 1997). Senescence involves the cessation of photosynthesis and complete breakdown as well as subsequent mobilization of leaf cellular materials (Buchanan-Wollaston, 1997). In addition, the process of senescence also brings about remobilization of vegetative N to supply substantial amounts to the seed in soybean (Jeppson et al., 1978; Zeiher et al., 1982; Staswick, 1994). However, seed nutritional composition may not be influenced by defoliation alone. In soybeans, seed protein concentration is an inherited quantitative trait that is influenced by the environment (Burton, 1987; 1989) and not as much by the embryo genotype or the plant genotype on which these seeds develop (Singh and Hadley, 1968). Therefore, whole plant processes like nitrogen fixation, translocation and C and N mobilization play an important role in determination of seed protein concentration (Burton et al., 1994).

The influence of leaf removal intensity on nutritional composition of harvested leaves or subsequent yield of three pulses, soybean, cowpea and lablab is not well documented. We conducted a study to i) determine the nutritional composition of leaves harvested at vegetative stage six (V6) from soybean, cowpea and lablab, ii) determine the optimum leaf harvesting percentage for legumes at V6 that does not compromise or negatively affect grain yield at

harvest, and iii) provide leaf harvest management recommendations to avoid plant stress and yield loss following leaf harvesting at V6.

Materials and methods

Experimental site

Field studies were conducted near Ames and Boone, Iowa in 2013 and 2014 respectively. In 2013, the field site and research studies were established at the Iowa State University Curtiss Farm (42°00'18.0"N 93°40'09.3"W). In 2014, the field study was done at the Agricultural Engineering and Agronomy Research farm (42° 1'18.76"N 93°46'35.94"W). Sites on both farms are drained by tiles and ditches due to poor drainage of excess precipitation (Hofstrand, 2010). Soil samples were collected before planting each year and samples were analyzed by the ISU Soil and Plant Analysis Laboratory (Table 1). The predominant soils at the Curtiss Farm site were Canisteo clay loam (Fine-loamy, mixed, superactive, calcareous, mesic Typic Endoaquolls) and Nicollet loam (Fine-loamy, mixed, superactive, mesic Aquic Hapludolls) soils. The soils at the Agricultural Engineering and Agronomy Research farm site were predominantly Canisteo silty clay loam and Clarion loam (Fine-loamy, mixed, superactive, mesic Typic Hapludolls) soils. Both sites had been in maize (*Zea mays* L.) – soybean rotation for several decades, with no known production history of cowpea or lablab in previous years.

Experimental design

The experimental design was a complete factorial of cultivars from three crop species and four leaf removal rates in a randomized complete block. In 2013, the pulse crops were soybean

'P92Y82' (DuPont Pioneer, Johnston, IA), cowpea 'Iron and Clay' (Corriher-olson et al., 2013) and lablab 'Rongai'(Wilson et al., 1962). In 2014, the pulse crops were soybean Pioneer 'P92Y82', cowpeas 'CA46' and 'Top Crop', and lablab 'Rongai'. Each site had 3 complete blocks with each block consisting of 12 treatments corresponding to leaf removal percentages. Plot size was 3.1-m wide by 7.6-m long with 30-inch row spacing. The seeding rate was 34 pure live seeds m^{-2} and the plots were planted on June 13th, 2013 and June 6th, 2014. Planting depth was about 2.5 cm.

Crop management

Fertilizer was not applied on site at the time of the experiment because available nutrient concentrations were adequate for soybean production in Iowa (Mallarino et al., 2013). A pre-plant application of pendimenthelin (N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine) in 187 L water ha^{-1} was done 2013 and 2014. Residual weed management was accomplished with a combination of cultivation, hand hoeing and hand pulling. In 2013, a weed infestation later in the season in soybean 'P92Y82' plots was controlled by glyphosate [N-(phosphonomethyl)glycine] [3.36 kg a.i. ha^{-1} in 93 L H_2O. Lambda-cyhalothrin ([1α(S*),3α(Z)]-(±)-cyano-(3-phenyloxyphenyl)methyl-3-(2-chloro-3,3,3,-tricfluoro-1-propenyl)-2,2-dimethylcycloprpanecarboxylate) was used to control Japanese beetles (*Popillia japonica*) in 2013 and leafhoppers (*Empoasca fabae*) in 2013 and 2014 early in the season. The insecticide was applied 9.08 g a.i. ha^{-1} in 93 L H_2O with a 0.762m open boom sprayer. The experiment relied solely on natural rainfall and plots were never irrigated.

Data Collection

Stand counts were taken when plants were between V4 and V6 stages using a randomly placed 5.3-m measuring rod along the length of each row. Weekly phenological data was collected from the two middle rows of each plot. Leaves were harvested at V6. Within each block, three/four plots had 99% of the leaves harvested at V6 vegetative stage six (V6) corresponding to six leaves (2013/2014), three/four plots had 66% of the leaves harvested corresponding to four leaves, three/four plots had 33% of the leaves harvested corresponding to two leaves and three/four control plots had 0% leaf removal. The leaves were hand harvested from 11.6 m^2 from the two center rows of each plot. Harvested into labelled paper bags and placed in the shade, the hand-harvested leaves were then dried at 60^o C in a forced-air oven. The leaves in the paper bags were turned regularly to prevent molding. Dried leaves were weighed and then ground to pass a 2-mm sieve using a rotary mill. Subsamples of the ground leaves were then taken to the Iowa State University Soil and Plant Analysis Laboratory for determination of total N, P, K, Mg, Ca, Mn, Fe, Cu, Zn, S and NO_3-N. Crude protein was calculated as N concentration × 6.25.

Aboveground biomass was determined at R5.5, prior to leaf loss, by hand-clipping 1 m-row from each plot. Samples were placed in a forced air oven at 60^o C until dry, and then weighed and ground to pass a 1-mm sieve. At developmental stage R8, the pulses were hand harvested for subsequent determination of yield components. Pods were hand harvested from 2-m of row from the two central rows of each plot. Plots were harvested on 10 and 17 October in 2013 and 2014, respectively. The pods were counted as they were taken off each stem and the number of pods m^2 calculated. Pods were then threshed with a stationary thresher (Model Almaco BT-14, Nevada, IA). Seed were counted using a Seedburo 801 Count-A-Pak (Seedburo

Equipment, Des Plaines, IL) seed counter. The seeds were weighed using a Balanza OHaus Explorer scale (Model E1B120, Parsippany, NJ) after drying in an oven over night at 60ºC.

Chemical and nutritional analysis

The concentration in leaves of P, K, Mg, Ca, Mn, Fe, Cu, Zn, and S was determined using microwave assisted nitric acid digestion followed by quantification with a Spectro Ciros ICP-OES. Nitrate-N was determined using a 2 M KCl extraction and cadmium reduction method on a Lachat QuikChem 8000 FIA+ (Pella, 1990; Horneck and Miller, 1998). Crude protein, oil and fiber analyses were done for soybean 'P92Y82' grain using Near Infrared Spectroscopy (NIRS). A subsample of cowpea seed from each plot was ground through a 1.0-mm sieve with a cyclone mill and used for total N determination using colorimetric procedures (LECO St. Joseph, MO). Crude protein was calculated as N × 6.25. Leaf samples were ground to pass a 1-mm sieve using a rotary mill for fiber analyses. Neutral and acid detergent fiber (NDF and ADF) and ash were done using ANKOM procedures (ANKOM Technology, Macedon, NY).

Results

Nutrient concentrations, 2013

The rate of defoliation/leaf removal impacted calcium concentration (Table 3). Calcium concentration in the dry leaves increased with increase in leaf removal percentage. The percentage of leaf removal did not significantly impact concentrations of crude protein, P, K, Mg, Mn, Fe, Cu, Zn or S in removed pulse leaf tissue in 2013 (Table 3). Although the concentrations for the above were not affected by the interaction of crop and leaf removal

percentage, some of them differed significantly by crop (Table 3). Soybean ‘P92Y82’leaves had the greatest concentration of crude protein whereas ‘Iron and Clay’ and Lablab leaves had similar concentrations. Soybean ‘P92Y82’ leaves contained 7.5 and 12% more crude protein than ‘Iron and Clay’ and lablab leaves, respectively. The P concentration differed significantly by crop. Lablab leaves contained the highest concentration of P, followed by ‘Iron and Clay’ and soybean ‘P92Y82’ leaves contained the lowest. Lablab leaves contained 10 and 17% more P than ‘Iron and Clay’ and soybean ‘P92Y82’ leaves, respectively. The concentration of Mg also differed significantly by crop. Soybean ‘P92Y82’ and lablab leaves had similar concentrations of Mg and both were greater than that for lablab. Soybean ‘P92Y82’leaves contained 28% more Mg than lablab whereas ‘Iron and Clay’ leaves contained 27% more Mg than lablab. Calcium concentration was statistically different among crops; ‘Iron and Clay’ leaves contained the greatest concentration whereas soybean ‘P92Y82’ leaves contained the least. ‘Iron and Clay’ leaves contained 47 and 15% more Ca than soybean ‘P92Y82’ and lablab leaves. In addition, leaf removal percentage also affected leaf calcium concentration significantly. Plants at the 99% leaf removal rate had the highest concentration of Ca in leaves whereas plots at 33% leaf removal had the lowest. Leaves from plots at 99% leaf removal contained 6 and 16% more calcium than leaves from plots at 66% and 33% leaf removal. The concentration of Mn was statistically the same in soybean ‘P92Y82’ and lablab leaves although it differed in ‘Iron and Clay’. ‘Iron and Clay’ leaves contained 58 and 56% more Mn than soybean ‘P92Y82’ and lablab. The concentration of S also differed significantly by crop. Lablab leaves contained the greatest concentration of S with 15 and 22% more S than ‘Iron and Clay’ and soybean ‘P92Y82’ leaves. The concentrations of K, Fe, Cu and Zn did not significantly differ by crop and neither

were they significantly affected by leaf removal percentage. Nitrate concentration was highest in soybean 'P92Y82' and 'Iron and Clay' leaves and lowest in lablab (Table 3).

Nutrient content,2013

Leaf removal percentage had significant impact on P content. The 99% leaf removal rate yielded 22 and 51% more P mg m^{-2} than 66 and 33% leaf removal respectively. P content also differed significantly by crop and lablab contained more P mg m^{-2} than soybean 'P92Y82' and 'Iron and Clay'. Lablab had 23 and 35% more P mg m^{-2} soybean 'P92Y82' and 'Iron and Clay' respectively. K content was also significantly affected by leaf removal (Table 4). K content was greatest at 99% leaf removal and lowest at 33%. Plots at 99% leaf removal had K content of 50 and 27% than those at 33 and 66% leaf removal respectively. K content was not significantly different amongst the crops. The Mg content significantly differed with leaf removal rate although no statistical differences were observed amongst the crops. Mg content was greatest at 66 and 99% leaf removal and lowest at 33% leaf removal. The Mg content at 66% leaf removal was 42% more than that 33% whereas mg content at 99% leaf removal was 50% more than that at 33% leaf removal (Table 4). The content of Mn was significantly affected by the rate of leaf removal (Table 4). The Mn content at 99% leaf removal was 65 and 30% more than that at 33 and 66% leaf removal. However, Mn content was not significantly different amongst crops. S content was significantly influenced by leaf removal rate and crop (Table 4). Leaf removal at 99% yielded 51 and 21% mg m^{-2} than 33 and 66% leaf removal. Lablab leaves had the greatest S content whereas 'Iron and Clay' leaves had the lowest. Lablab leaves contained 39 and 28 % more S mg m^{-2} than 'Iron and Clay' and soybean 'P92Y82' leaves respectively. The Ca content was also significantly affected by leaf removal percentage and differed significantly amongst

crops. Leaf removal at 99% yielded 58 and 25% more Ca mg m^{-2} than 33 and 66% leaf removal respectively. Lablab leaves had the greatest calcium content whereas soybean 'P92Y82' had the lowest. Lablab leaves contained 43 and 17% more Ca mgm^{-2} than soybean 'P92Y82' and 'Iron and Clay' respectively. The Fe, Cu and Zn contents were neither significantly affected by leaf removal percentage nor did they differ statistically by crop. However, Nitrate content was highest in soybean 'P92Y82' and 'Iron and Clay' and lowest in lablab leaves (Table 4).

Plant stand, aboveground biomass, grain yield, and fiber concentrations 2013

Leaf removal percentage did not significantly affect plant stand at V4 and R8. However, stand density was significantly different amongst crops (Table 5). At V4, 'Iron and Clay' cowpeas had the greatest stand density whereas soybean 'P92Y82' had the lowest. The stand density at V4 in 'Iron and Clay' plots was 46 and 30% more soybean 'P92Y82' and lablab. At maturity R8, stand density was similar to that of lablab and 'Iron and Clay' but it was greater than for soybean 'P92Y82'. The stand density at R8 for lablab was 35% greater than that of soybean 'P92Y82' whereas the stand density at R8 in 'Iron and Clay' plots was 35% greater than that for soybean 'P92Y82'.

Leaf removal percentage significantly affected the amount of dry leaves per hectare and the 99% leaf removal rate had the driest leaf biomass harvested on an area basis whereas plots at 33% leaf removal had the lowest. Leaf harvesting at 99% yielded 20 and 49% more leaves per hectare than 66 and 33% leaf removal. However, the amount of leaf material harvested on an area basis did not differ significantly by crop. Aboveground biomass accumulation was not influenced by leaf removal rate or by crop (Table 5).

Grain yield differed significantly by crop but not by leaf removal rate (Table 5). Soybean ‘P92Y82’ yielded 99% more grain than ‘Iron and Clay’. Lablab did not produce any grain. The main effect of leaf removal rate did not significantly impact the number of pods per square meter, number of seeds per pod, the number of seeds per square meter or weight per seed (Table 5). However, these factors differed significantly by crop. Soybean ‘P92Y82’ seeds weighed 60% more than ‘Iron and Clay’ seeds. Soybean ‘P92Y82’ plants had 98% more seeds per square meter than ‘Iron and Clay’. However, ‘Iron and Clay’had 63% more seeds pod^{-1} than soybean ‘P92Y82’ although soybean ‘P92Y82’ had 99% more pods m^{-2} than ‘Iron and Clay’ (Table 5). Soybean ‘P92Y82’ height at maturity was not significantly affected by leaf removal percentage.

Fiber concentration differed significantly by crop (Table 6). However, leaf removal percentage did not significantly impact NDF, ADF, ADL, hemicellulose, cellulose or ash concentration in the leaves and neither did the interaction of leaf removal percentage and crop (Table 6). The NDF concentration was greatest in soybean ‘P92Y82’ and lablab leaves and lowest in ‘Iron and Clay’. NDF concentration in soybean ‘P92Y82’ leaves was 10% more than that in ‘Iron and Clay’ whereas this concentration in 8% more in lablab leaves than ‘Iron and Clay’. The ADF concentration also was highest in soybean ‘P92Y82’ and lablab and least in ‘Iron and Clay’. The ADF concentration in soybean ‘P92Y82’ leaves was 11% more than that in ‘Iron and Clay’ whereas this concentration in lablab was 7% more than that in ‘Iron and Clay’ (Table 6). The ADL concentration was highest in soybean ‘P92Y82’ and lablab and lowest in ‘Iron and Clay’. The ADL concentration in soybean ‘P92Y82’ was 21% more than that in ‘Iron and Clay’ whereas this concentration in lablab was 16% more than that in ‘Iron and Clay’ leaves. Hemicellulose was highest in soybean ‘P92Y82’ and lablab leaves and lowest in ‘Iron and Clay’. The concentration of hemicellulose in soybean ‘P92Y82’ was 7% than that in ‘Iron and Clay’

whereas this concentration in lablab was 12% more than that in 'Iron and Clay' leaves. The concentration of cellulose was highest in soybean 'P92Y82', followed by lablab leaves and lowest in 'Iron and Clay' leaves. Soybean 'P92Y82' leaves contained 4 and 8% more cellulose than lablab and 'Iron and Clay' leaves respectively. The concentration of ash was not significantly affected by leaf removal percentage and neither did it differ significantly amongst the crops (Table 6).

Nutrient concentrations, 2014

Leaf removal rate influenced Mn concentration of removed leaves (Table 7). The highest concentration of Mn in removed leaves was observed when leaf removal was done at 99% removal. The concentration of Mn at 99% leaf removal was 31 and 47% more than that 66 and 33% leaf removal respectively. In addition, this concentration differed significantly among soybean 'P92Y82', 'CA46' and 'Top Crop' cowpea and lablab. 'Top Crop' and 'CA46' cowpeas had the highest Mn concentration and did not differ from each other; soybean 'P92Y82' and lablab had the lowest Mn concentration in removed leaves. 'CA46' leaves contained 50 and 51% more Mn than soybean 'P92Y82' and lablab, respectively, whereas 'Top Crop' contained 41 and 42% more Mn than soybean 'P92Y82' and lablab leaves respectively. The concentration of Fe was significantly affected by crop and also differed statistically among the four crops. Leaf removal at 99% yielded 38 and 65% more Fe than 66 and 33% leaf removal. Lablab leaves had the highest Fe concentration whereas soybean 'P92Y82' and 'Top Crop' had the lowest. Lablab leaves contained 33, 42 and 50% more Fe than 'CA46', soybean 'P92Y82' and 'Top Crop' leaves respectively (Table 7). The concentrations of Mg and Ca were not significantly affected by leaf removal percentage; however, these nutrients differed significantly amongst crops. The

concentration of Mg was highest was highest in soybean ‘P92Y82’ and ‘CA46’ leaves and lowest in lablab. Soybean ‘P92Y82’ leaves contained 19 and 51% more Mg than ‘Top Crop’ and lablab leaves whereas ‘CA46’ leaves contained 8 and 44% more Mg than ‘Top Crop’ and lablab leaves respectively. Calcium concentration was highest in ‘Top Crop’ leaves and lowest in soybean ‘P92Y82’ leaves. ‘Top Crop’ leaves contained 23, 24 and 32% more calcium than ‘CA46’, lablab and soybean ‘P92Y82’ respectively. Leaf removal percentage had no significant impact on the concentration of P, K, Cu, Zn, S and CP and neither did these nutrients differ significantly by crop. Leaf removal percentage did not significantly impact nitrate concentration; however, concentration of nitrate significantly differed by crop. Nitrate concentration was greatest in ‘CA46’ leaves and lowest in lablab (Table 7).

Nutrient content, 2014

The content of P, K, Mg, Ca, Mn, Fe, Cu, Zn, S and nitrate in leaves differed significantly with leaf removal percentage. In addition, the content of these elements was also statistically different amongst crops. At 66 and 99% leaf removal harvested leaves provided 51 and 50% more P mg m^{-2} in leaves from the 33% leaf removal rate (Table 8). In addition, ‘CA46’ leaves had the greatest content of P. ‘CA46’ leaves contained 12, 27, 30% more P mg m^{-2} than ‘Top Crop’, lablab and soybean ‘P92Y82’ leaves. The content of K in leaves also differed significantly with the leaf removal percentage. Leaves from plots at 99 and 66% leaf removal contained 55 and 58% more K than those at 33% respectively. ‘CA46’ leaves contained 18, 19 and 48% more K than soybean ‘P92Y82’, ‘Top Crop’ and lablab leaves respectively. The Mg content was highest in leaves at 66 and 99% leaf removal and lowest at 33% (Table 8). Leaves from 99 and 66% leaf removal rates yielded 59% more Mg than those at 33%. However, Mg

content was similar for soybean ‘P92Y82’, ‘CA46’ and ‘Top Crop’ leaves and lowest in lablab. The Ca content was significantly affected by leaf removal percentage (Table 8). Leaf removal at 99% yielded 19 and 71% more Ca than 66 and 33% leaf removal. Ca content also differed significantly by crop (Table 8). The Ca content in ‘CA46’ and ‘Top Crop’ leaves was similar and both were greater for Ca content than soybean ‘P92Y82’ and lablab leaves. The content of Mn in leaves at 99% leaf removal was 33 and 78% more than that at 66 and 33% leaf removal. ‘CA46’ contained 22, 56 and 67% more Mn mg m^{-2} than ‘Top Crop’, soybean ‘P92Y82’ and lablab leaves. Fe content was significantly affected by leaf removal percentage. Leaf removal at 99% yielded 37 and 84% more Fe than 66 and 33% leaf removal rates respectively. Fe content did not significantly differ among crops. The content of Cu was significantly affected by leaf removal rate. Leaves from plots at 66 and 99% leaf removal had the same Cu content and this was greater than content in leaves at 33% leaf removal. Cu content also significantly differed amongst the crops. ‘CA46’ leaves had the highest content whereas soybean ‘P92Y82’ and lablab had the lowest Cu content. ‘CA46’ leaves contained 20, 34 and 44% more Cu mg m^{-2} than ‘Top Crop’, soybean ‘P92Y82’ and lablab leaves respectively (Table 8). Zinc content was significantly affected by leaf removal percentage although this content did not statistically differ among the crops. Leaf removal at 66% yielded 42 and 61% more Zn content in harvested leaf removal than 99 and 33%. The S content was significantly affected by leaf harvesting percentage and differed statistically by crop. Leaf removal at 99 and 66% resulted in greater S yield than the 33% leaf removal rate. ‘CA46’ leaves had the highest content of S whereas lablab leaves had the lowest. ‘CA46’ leaves contained 18, 12 and 33% more S than soybean ‘P92Y82’, ‘Top Crop’ and Lablab leaves respectively. Nitrate content differed significantly with different leaf removal percentages. This content was highest at 66% leaf removal and lowest at 33%. Nitrate content

also differed statistically among crops. ‘CA46’ leaves had the greatest content of crude protein whereas, ‘Top Crop’ and lablab leaves contained the least. ‘CA46’ leaves contained 44, 56 and 81% more crude protein soybean ‘P92Y82’, ‘Top Crop’, and lablab leaves respectively (Table 8).

Plant stand, aboveground biomass, grain yield, and fiber concentrations, 2014

Although leaf removal rate and/or crop influenced agronomic and leaf fiber characteristics, the interactions of leaf removal rate × crop was never significant (Tables 9 and 10). Leaf removal percentage had no significant impact on plant stand at V4 or R8. In addition, plant stand at R8 did not differ significantly by crop (Table 9). However, at V4, stand density was statistically different amongst soybean ‘P92Y82’, ‘CA46’, ‘Top Crop’ and lablab. The stand density at V4 for ‘CA46’ and ‘Top Crop’ was not statistically different and neither was that of soybean ‘P92Y82’ and lablab. This stand density was highest in ‘CA46’ and ‘Top Crop’ and lowest in soybean ‘P92Y82’ and lablab. There were no statistical differences in aboveground biomass at 0, 33, 66 and 99% leaf removal. However, biomass was significantly different amongst the crops. Soybean ‘P92Y82’ had 27, 45 and 46% more biomass than ‘CA46’, ‘Top Crop’ and lablab. Soybean ‘P92Y82’ had the highest amount of aboveground biomass followed by ‘CA46’. ‘Top Crop’ and lablab had similar aboveground biomass, the least of the four crops (Table 9).

Plants that did not have leaves removed had the greatest grain yield whereas plants at 66 and 99% leaf removal yielded the least (Table 9). Plants at 0% leaf removal had 20, 32 and 35% more yield than those subjected to 33, 66 and 99% leaf removal respectively. Grain yield also differed significantly by crop. Soybean ‘P92Y82’ had 64 and 72% more yield that ‘CA46’ and

'Top Crop' respectively. Leaf removal did not influence pod density (Table 9). However, the number of pods differed significantly among the crops. Soybean 'P92Y82' had 80 and 79% more pods than 'CA46' and 'Top Crop' respectively. Pod density was similar for the two cowpeas and averaged 158 m^{-2}. Grain yield was significantly affected by leaf removal percentage.

The number of seeds per pod did not differ significantly for leaf removal rate, however seed per pod differed among crops (Table 9). 'CA46' and 'Top Crop' cowpeas had similar number of seeds per pod while soybean 'P92Y82' had the lowest number of seeds per pod. 'CA46' had 53% more seed per pod than soybean 'P92Y82' whereas 'Top Crop' had 43% more seeds per pod than soybean 'P92Y82'. The numbers of seeds m^{-2} were significantly affected by leaf removal rate (Table 9). This parameter also differed among crops. Soybean 'P92Y82' yielded the most number of seeds per square meter. 'Top Crop' and 'CA46' seeds per square meter did not significantly differ. Leaf removal rate influenced seed number m^{-2}. The no- leaf removal control yielded more seeds than leaf removal rates of 33, 66 and 99%. Plants at 0% leaf removal had 17% seeds m^{-2} more than those with 33% leaf removal, 28% more seeds m^{-2} than those at 66% and 99% leaf removal. Seed weight also statistically differed by crop (Table 9). Soybean 'P92Y82' seeds weighed 21 and 29% more than 'CA46' and 'Top Crop' seeds respectively (Table 9). The rate of leaf removal did not significantly impact seed crude protein concentration. Soybean 'P92Y82' had the highest amount of crude protein, followed by 'CA46' and 'Top Crop' with the lowest concentrations (Table 9). Soybean 'P92Y82' seeds contained 25 and 30% more crude protein than seed of 'CA46' and 'Top Crop' cowpeas. Seed weight differed significantly with different leaf removal percentage and amongst crops (Table 9). Seeds from plants at 0% leaf removal weighed more than seeds from plots at 33, 66 and 99% leaf removal.

Seeds from plots that were not leafed weighed 7, 11 and 12% more than seeds from plots at 33, 66 and 99% leaf removal.

The leaf removal rate did not influence concentrations of NDF, hemicellulose and dry matter in soybean 'P92Y82', 'CA46', 'Top Crop' and lablab and neither did these concentrations differ amongst the crops (Table 10). Leaf removal percentage had no significant impact on ADF concentration in leaves. However, this concentration in leaves differed significantly amongst the crops. Soybean 'P92Y82' had the highest content of ADF and lablab the lowest. ADF concentration in leaves was not significantly different between 'CA46' and 'Top Crop'. Soybean 'P92Y82' leaves contained 25 and 27% more ADF than the two cowpeas and lablab respectively (Table 10). The ADL concentration was significantly affected by leaf harvesting rate and crop but the interaction of leaf harvesting rate × crop was not significant (Table 10). Leafs from the 66% removal rate had 3 and 26% more ADL than leaf removal at 99 and 33%. Soybean 'P92Y82' leaves also contained the greatest amount of ADL whereas 'CA46' contained the lowest. The ADL concentration in 'Top Crop' and lablab leaves was not statistically different. Soybean 'P92Y82' leaves contained 24 and 11% more ADL than 'CA46', 'Top Crop' and lablab respectively. Cellulose concentration did not significantly differ by leaf removal rate (Table 10). Mean cellulose concentration for 33, 66 and 99% was 132 g kg^{-1}. Leaf cellulose concentration differed among pulse crops (Table 10). Soybean 'P92Y82' had the greatest concentration of cellulose and lablab had the lowest. Soybean 'P92Y82' leaves contained 25% more cellulose than 'CA46' and 'Top Crop' and 30% more than lablab leaves. Leaf removal percentage had significant impact on ash concentration and ash concentration also differed significantly amongst the crop leaves. Soybean 'P92Y82' had the highest concentration of lignin. Soybean 'P92Y82' leaves had 24% more lignin concentration than 'CA46' and 'Top Crop' and 8% more than

lablab. The concentration of ash was significantly affected by leaf removal percentage and also differed statistically by crop. Leaves from plots at 66 and 99% leaf removal had the highest concentration of ash whereas those at 33% leaf removal had the lowest. Lablab leaves contained 82, 73 and 63% more ash than 'Top Crop', 'CA46' and soybean 'P92Y82' leaves, respectively (Table 10).

Discussion

The rate of leaf removal impacted calcium, iron and manganese concentrations in the removed leaves. These concentrations in dry leaves increased with increase in leaf removal percentage. This may be because they are non-mobile elements and therefore deficiencies occur in newer leaves/tissues and therefore concentration increases with the age of the leaves (Owen and Kissel, 2015). This may explain why the higher percentage of leaf removal from top (younger leaves) moving down to older leaves resulted in higher concentration of these nutrients at 99 and 66% leaf removal compared to 33% leaf removal. Similarly, Lincoln et al. (2010) explained that although younger leaves have greater need for calcium, older leaves contained higher amounts because of relatively immobility of calcium through the plant. The authors explained that calcium in particular is not transported through the phloem since it can bind with other nutrient ions and precipitate out of liquid solutions.

Most of the leaf nutrient concentrations differed significantly by crop. The rate and magnitude of change in nutritive value differed according to species, soil fertility, growing environment and many others. This was reported previously by Press (2011) who found differences in nutritive value of leaves by crop or plant species. Although other factors such as climate and soil factors could have significant influence on leaf nutrient composition, plant

species is the most important influence on leaf nutritional composition (Press, 2011). Daniel (1934) found that certain crop species were usually high in some nutrients like calcium and phosphorous even when grown on soils that were relatively low in some minerals. The findings suggest that genetic characteristics inherent to specific plants may have great influence on leaf nutritional composition (Press, 2011). However, it is not quite clear why leaf removal did not significantly affect fiber concentrations in 2014 although it led to significant differences in ADL and ash in 2013.

Soybean 'P92Y82' had the greatest amount of aboveground biomass compared to cowpea and lablab. This corresponds with Rao and Northup (2009) who reported that soybean produced more biomass than cowpea for three years in a four year experimental study in Oklahoma The amount cowpea biomass also corresponds with previous studies by Agza et al. (2012) who reported dry matter accumulation yields of different cowpea genotypes ranging between 2330 kg ha^{-1} to 7670 kg ha^{-1}. Environment and Muchow (1985) further explained the differences in biomass among different crops. The authors reported that biomass production was a function of incident solar radiation, the proportion intercepted, the mode of interception and the respiratory as well as photosynthetic characteristics of the crop. They added that proportion of radiation intercepted is determined by the size and arrangement of leaf canopy whereas the mode of interception is a factor determined by leaf orientation within the canopy. These factors differ among soybean 'P92Y82', lablab, 'CA46', 'Top Crop' and 'Iron and Clay' and may have likely caused the differences in aboveground biomass by crop.

Yield data were collected for only soybean 'P92Y82', 'Iron and Clay', 'CA46' and 'Top Crop' and none on lablab. Lablab did not reach physiological maturity and therefore no yield data was collected. Lablab 'Rongai' is a short-day plant that is quite sensitive to day length and

flowers best with less than 11 hours of day light although it was reported to require ample sunlight. Day length in Ames, Iowa was between 16 to 12 hours 2013 and 2014 growing season. Lablab 'Rongai' usually seeds late and has low frost tolerance (FAO, 2015). However, yield differed significantly by crop. Soybean 'P92Y82' had more yield than the three cowpeas in 2013 and 2014. This could be because plant breeders have done immense work with soybean in Iowa for over 60 years but none on breeding cowpea or lablab in Iowa. In addition, soybean 'P92Y82' had more pods per square meter and also because soybean 'P92Y82' seeds weighed more than 'CA46', 'Top Crop' and 'Iron and Clay' seeds. Deshmukh et al. (2011) explained that in cowpeas, the overall yield depended upon the number of pods and size of the seed. These two factors are genetically determined (Willmer and Johnston, 1976; Atkins et al., 1977). Soybean has a higher yield potential in this environment and that could be one of the reasons why farmers in Ames, Iowa grow more soybean than cowpea. In addition, Bhuvaneswari et al. (1988), reported that cowpeas had a higher threshold of response to signal substances from the bacteria for nitrogen fixation than soybeans. This in turn could affect their yield compared to soybeans. In addition, environmental factors such as temperatures significantly influence flower and pod shedding in both cowpeas and soybean (Van Schaik et al., 1958; Gibson and Mullen, 1996; Warrag and Hall, 1984) and with consequent effects on yield. Greater soybean yield compared to the cowpeas may be attributed biomass since soybean 'P92Y82' had more biomass than the cowpeas in both years. Soybean yield can be determined as a product of biomass and harvest index and Spaeth et al. (1984), explained that holding harvest index as a constant, high biomass would produce high yields. Findings by Spaeth et al. (1984) are consistent with those of Duncan (1986) who reported that total dry matter was directly proportional to yield especially when total dry matter was produced before the seed initiation process. However, yield is not only affected

by biomass accumulation but also by other factors such as genetics, planting date, diseases, and crop management (Conley et al., 2015)

Although leaf harvesting percentage had no significant impact on yield in 2013, leaf removal significantly affected grain yield in 2014. The absence of statistical differences for yield in 2013 may be because leaf removal did not affect the source sink relationship that year. Significant reduction in yield with leaf removal percentage in 2014 on the other hand, may be because of reduction in source leaves that limited the reproductive sink size. This corresponds with findings by Bubenheim and Nielsen (1990). Hoogesteger and Karlsson (1992) also reported that leaf removal altered photosynthesis directly through changing source-sink relations because when leaves were harvested, photosynthates were directed towards development of new leaves at the expense of being used in grain production. Since the experiment was done in two years and at a different site each year, differences in climate, soil factors, and plant nutrition, may account for differences in source sink relations of the plants in each of the two years.

Soybean 'P92Y82' yielded more than 'CA46', 'Top Crop' and 'Iron Clay' but the cowpeas had more seeds per pod than soybean 'P92Y82'. These differences in seed weight may be attributed to differences in variety and genetic makeup of the crops (Sene, 1968; Aryeetey and Laing, 1973; Drabo et al., 1973). In 2013, 'Iron and Clay' produced very few seeds (of very small size) to no seeds and therefore analysis for crude protein was not done that year to determine effects of leaf removal among soybean 'P92Y82' and 'Iron and Clay'. In 2014, soybean 'P92Y82' seeds contained more crude protein than 'CA46' and 'Top Crop' seeds. This corresponds with reports by Awolumatea (1983) who found that, although cowpeas accumulated nitrogen at a rate much faster than soybean during seed development, 994 $\mu g\ day^{-1}$ compared to 473 $\mu g\ day^{-1}$ respectively, crude protein of biomass decreased with development time from 40%

in early seed development stages to 26% in mature cowpea and from 35 to 33% in soybeans. The greater decrease in cowpea crude protein at maturity compared to soybean could explain why soybean 'P92Y82' seeds contained more crude protein than cowpeas. The non-significant effects of leaf removal rate on seed crude protein in 2014 are in line with findings by Burton et al. (1995) and Lawn and Brun (1974) who reported unchanged seed protein content with leaf removal. Reduction in seed crude protein with leaf removal is attributed to reduction in vegetative N remobilization which causes low protein concentrations Burton et al. (1995). Non-significant effects of leaf removal on seed protein may be explained by compensatory regrowth which then covers vegetative N remobilization for seed protein.

Soybean 'P92Y82' leaves had the greatest concentration of crude protein whereas 'Iron and Clay' and Lablab leaves had similar concentrations. Soybean 'P92Y82' leaves contained 7.5 and 12% more crude protein than 'Iron and Clay' and lablab leaves respectively and P concentration differed significantly by crop. The crude protein from our study is slightly more than that found by Blount et al., 2009. This may be because in their study, leaves for dry matter analysis of crude protein were picked at 50% bloom. They found crude protein in soybean leaves to be 178 g kg^{-1}. Lablab leaves contained the highest concentration of P, followed by 'Iron and Clay' and soybean 'P92Y82' leaves contained the lowest. Lablab leaves contained 10 and 17% more P than 'Iron and Clay' and soybean 'P92Y82' leaves respectively. Lower crude protein in 'Iron and Clay' and lablab leaves compared to soybean 'P92Y82' may be attributed to poor inoculation of the plants hence N deficiency for crude protein in leaves. Additional studies could examine for other nutrients such as folate in pulse legume leaves would be beneficial. Folate for instance is an important compound for cell division and formation of DNA and deficient in many

women in both developed and developing countries yet is known to be available in green leafy vegetables.

Conclusion

The utilization of soybean, cowpea and lablab leaves as leafy vegetables or forage may improve human and ruminant nutritional by using leaves that otherwise be left in the field. For humans, the leaves may be a potential source of very important micronutrients such as iron and zinc and dietary fiber. The leaves could be an excellent source of crude protein for livestock. Therefore, supplementary nutritional analysis of the leaves for folate and similar nutrients will be beneficial. In addition, efficient leaf harvesting methodology is necessary and would be beneficial to aid the process.

Acknowledgements

The authors are grateful for technical assistance from Gary Hammitt, Danielle Wilson, Luke Hodnefield and Roger Hintz and the help of several graduate and undergraduate students in the field and the laboratory.

REFERENCES

Aryeetey, A.M., Laing, E., 1973. Inheritance of yield components and their correlation with yield in cowpea (*Vigna unguiculata* L. Walp.). Euphytica. 22, 386-392.

Atkins, C.A., Kuo, J., Pate, J.S., Flinn, A.M., Steele, T.W., 1977. Photosynthetic pod wall of pea (*Pisum sativum* L.), distribution of carbon-fixing enzymes in relation (*Pisum sativum* L.), distribution of carbon-fixing enzymes in relation to pod structure. Physiol. 60, 779-786.

Awolumatea, E., 1983. Accumulation and quality of storage protein in developing cowpea , mung bean and soya bean seeds. J. Sci. Food. Agri. 34, 1351–1357.

Badi, S.H., Dikwahal, H.D., Jibung, G.G., 2012. Response of vegetable cowpea (*Vigna unguiculata* (L.) Walp.) to intra-row spacing and defoliation at Garkawa. Asian J. Agric. Sci. 4, 210–212.

Baloyi, B.M., Ayodele, V.I., 2013. Effects of leaf harvest on crude protein and mineral contents of selected early maturing lines of lablab (*Lablab purpureus*). Afr. J. Agric. Res. 8, 449–453.

Bayne, H.G., Brown, M.S., Bethlenfalvay, G.J., 1984. Defoliation effects on mycorrhizal colonization , nitrogen fixation and photosynthesis in the *Glyicne-Glomus-Rhizobium* symbiosis. Physiol. Plant. 62, 576–580.

Blount, A.R.S., Wright, D.L., Sprenkel, R.K., Hewitt, T.D., Myer, R.O., 2009. Forage soybeans for grazing , hay and silage. University of Florida IFAS Extension. Publication #SS-AGR-1801,1-5. https://edis.ifas.ufl.edu/ag184 (accessed 30 Nov. 2015).

Board, J.E., Wier, A.T., Boethel, D.J., 1994. Soybean yield reductions caused by defoliation during mid to late seed filling. Agron. J. 86, 1074–1079.

Brkić, S., Milaković, Z., Kristek, A., Antunović, M., 2004. Pea yield and its quality depending on inoculation , nitrogen and molybdenum fertilization. Plant Soil. Environ. 50, 39–45.

Browde, J.A., Pedigo, L.E., Owen, M.D.K., Tylka, G.L., Levene, B.C., 1994. Growth of soybean stressed by nematodes , herbicides , and simulated insect defoliation. Agron. J. 86, 968–974.

Burton, J.W., Israel, D.W., Wilson, R.F., Carter, T.E., 1994. Effects of defoliation on seed protein concentration in normal and high protein lines of soybean. Plant. Soil. 172, 131–139.

Corriher-Olson, V., Smith, G.R., 2013. Establishment of tropical annual legumes sod-seeded into bermudagrass or prepared seedbed. Tex. J. Agri. Nat. Resourc. 26, 73–81.

Demooy, B.E., Demooy, C.J. 1989. Effects of leaf-harvesting practices on yield and yield components of ER-7 cowpea (*Vigna unguiculata* (L.) Walp.) in semi-arid Botswana. Field Crops Res. 22, 27–31.

Deshmukh, D.V, Mate, S.N., Bharud, R.W., Harer, P.N., 2011. Analysis of pod and seed development in cowpea [*Vigna unguiculata* (L .) Walp]. Am. Eurasian. J. Agron. 4, 50–56.

Drabo, I., Redden, R., Smithson, J.B., Aggarwal, V.D., 1973. Inheritance of seed size in cowpea (*Vigna unguiculata* (L .) Walp.). Euphytica. 33, 929–934.

Ehlers, J.D., Hall, A.E., 1997. Cowpea (*Vigna unguiculata* (L.) Walp.). Field Crops Res. 53, 187–204.

Fening, J., Danso, S.K., 2002. Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. Appl. Soil. Ecol. 21, 23–29.

Figueiredo, M.V.B., Vilar, J.J., Burity, H.A., de França, F.P., Janeiro-rj, R.D., 1999. Alleviation of water stress effects in cowpea by *Bradyrhizobium* spp. inoculation. Plant. Soil. 207, 67–75.

Gazzoni, C.I.O.L., Moscardi, V.I.O., 1997. Effect of defoliation levels on recovery of leaf area , on yield and agronomic traits of soybeans. Braz. J. Agric. Res. 33, 411-424.

Gibson, L.R., Mullen, R.E., 1996. Influence of day and night temperature on soybean seed yield. Crop. Sci. 36, 98–104.

Grymes, C.F., Griffin, J.L., Boethel, D.J., Leonard, B.R., David, L., Russin, J. S., Jordan, D.L., 1999. Soybean response to weed interference and defoliation. Weed Sci. 47, 90–94.

Hammond, R.B., 1988. Effects of leaf removal at soybean growth stage V1 on yield and other growth parameters. J. Kans. Entomol. Soc. 62, 96–102.

Harrison, H.F., Thies, J.A., Fery, R.L., Smith, J.P., 2006. Evaluation of cowpea genotypes for use as a cover crop. Hortsci. 41, 1145–1148..

Hashem, F.M., Saleh, S.A., Berkum, P.V., Voll, M., 1997. Survival of *Bradyrhizobium* sp . (*Arachis hypogaea* L.) on fungicide-treated peanut seed in relationship to plant growth and yield. World J. Microbiol. Biotechnol. 13, 335–340.

Hikosaka, K., Takashima, T., Kabeya, D., Hirose, T., Kamata, N., 2005. Biomass allocation and leaf chemical defence in defoliated seedlings of Quercus serrata with respect to carbon-nitrogen balance. Ann. Bot. 95, 1025–1032.

Hintz, R.W., Beeghly, H.H., Fehr, W.R., Schneiter, A.A., Hicks, D.R., 1991.Soybean response to stem cutoff and defoliation during vegetative development. J. Prod. Agric. 4, 585–589.

Hirsch, P.R., 1996. Population dynamics of indigenous and genetically modified rhizobia in the field. New Phytol. 133, 159–171.

Hofstrand, J., 2010. Economics of tile drainage. https://www.extension.iastate.edu/agdm/articles/hof/HofJuly10.html (Accessed 1 Dec 2015).

Johnston, T.J., Pendleton, J.W., 1968. Contribution of leaves at different canopy levels to seed production of upright and lodged soybeans (*Glycine max* (L.) Merrill)1. Crop. Sci. 8, 291-292.

Kovalenko, I.V., Rippke, G.R., Hurburgh, C.R., 2006. Determination of amino acid composition of soybeans (*Glycine max*) by Near-Infrared Spectroscopy. J. Agric. Food Chem. 54, 3485-3491.

Klubertanz, T. H., Pedigo, L.P., Carlson, R.E., 1996. Soybean physiology, regrowth, and senescence in response to defoliation. Agron. J. 88, 577–582.

Kutcher, H.R., Lafond, G., Johnston, A.M., Miller, P.R., Gill, K.S., May, W.E., 2002. Rhizobium inoculant and seed-applied fungicide effects on field pea production. Can. J. Plant. Sci. 82, 645–661.

Law, I.J., Botha, W.F., Majaule, U.C., Phalane, F.L., 2006. Symbiotic and genomic diversity of "cowpea" bradyrhizobia from soils in Botswana and South Africa. Biol. Fertil. Soils. 43, 653–663.

Lovvorn, R. L., Smith, B.E.W., 1943. Response of soybeans to experimental defoliation. J. Am. Soc. Agron. 35, 768–778.

Maass, B.L., Knox, M.R., Venkatesha, S.C., Angessa, T.T., Ramme, S., Pengelly, B.C., 2010. *Lablab purpureus*-A Crop Lost for Africa? Trop. Plant. Biol. 3, 123–135.

Martins, L.M.V., Xavier, G.R., Rangel, F.W., Ribeiro, J.R.A., Neves, M.C.P., Morgado, L.B., Rumjanek, N.G., 2003. Contribution of biological nitrogen fixation to cowpea: a strategy for improving grain yield in the semi-arid region of Brazil. Biol. Fertil. Soils., 38, 333–339.

Mcalister, D.F., and Krober, O.A., 1958. Response of soybeans to leaf and pod removal. Agron. J. 50, 674–677.

Midega, C.A.O., Salifu, D., Bruce, T. J., Pittchar, J., Pickett, J. A., Khan, Z.R., 2014. Cumulative effects and economic benefits of intercropping maize with food legumes on *Striga hermonthica* infestation. Field Crops. Res. 155, 144–152.

Muthomi, J.W., Otieno, P.E., Chemining, G.N., Nderitu, J.H., 2007. Effect of root rot pathogens and fungicide seed treatment on nodulation in food grain legumes. J. Biol. Sci. 7, 1163–1170.

Ogedegbe S.A., Ongunlela, V.B., Olufajo, O.O., Odion, E.C., 2012. Seed yield and yield attributes of lablab as influenced by phosphorous application, cutting height. Asian. J. Crop. Sci. 4, 12–22.

Ohler, T.A., Nielsen, S.S., Mitchell, C.A., 1996. Varying plant density and harvest time to optimize cowpea leaf yield and nutrient content. Am. Soc. Hortic. Sci. 3, 193–197.

Oelberg, K., 1956. Factors affecting the nutritive value of range forage. J. Range. Manage. 9, 220–225.

Pickle, C.S., Caviness, C.E.,1983.Yield reduction from defoliation and plant cutoff of determinate and semideterminate soybean. Agron. J. 4, 474–476.

Proulx, R.A., Naeve, S.L., 2009. Pod removal, shade, and defoliation effects on soybean yield, protein, and oil. Agron. J. 101, 971–978.

Rao, S.C., Northup, B.K., 2009. Capabilities of four novel warm-season legumes in the southern great plains: Biomass and forage quality. Crop. Sci. 49, 1096–1102.

Saidi, M., Ngouajio, M., Itulya, F.M., Ehlers, J., 2007. Leaf harvesting initiation time and frequency aff ect biomass partitioning and yield of cowpea. Crop. Sci. 47, 1159–1166.

Schulz, T.J., Thelen, K.D., 2008. Soybean seed inoculant and fungicidal seed treatment effects on soybean. Crop. Sci. 48, 1975–1983.

Schulz, T.J., Thelen, K.D., Wang, D., 2005. The effect of *Bradyrhizobium japonicum* inoculant on soybean growth and yield. http://www.researchgate.net/publication/237651647_The_Effect_of_*cu_japonicum*_inoculant_on_soybean_growth_and_yield (Accessed 30 Nov. 2015).

Somasegaran, P., Hoben, H.J., 1985. Methods in legume-*Rhizobium* technology. University of Hawaii NifTAL. Hawaii Institute of tropical agriculture and human resources.

Teigen, J.B., Vorst, J.J., 1975. Soybean response to stand reduction and defoliation. Agron. J. 67, 813–816.

Van Kessel, C., Hartley, C., 2000. Agricultural managemnet of grain legumes: has it led to an increase in nitrogen fixation? Field Crops Res. 65, 165-181.

Wood, I.,1983. Lablab bean (*Lablab purpureus*) for grain and forage production in the Ord irrigation area. Aust. J. Exp. Agric. 23, 121-162.

Table 1.

Pre-plant soil concentrations for available P, K, Nitrate-N, pH and OM for two years.

Year	Depth	M_3P mg kg^{-1}	M3K mg kg^{-1}	pH	OM g kg^{-1}	Nitrate-N mg kg^{-1}	EC dS m^{-1}
2013	0 - 15 cm	35	198	5.7	4.6	4	1.152
2013	15 - 30 cm	7	136	6.5	4.2	5	0.35
2014	0 -15 cm	43	153	5.5	3.6	7	-
2014	15 - 30cm	14	99	5.6	2.7	4	-

- data was not collected

Table 2

Long-term monthly average air temperature and total precipitation during the two-year study.

Month	2014	2013	Long-term (12 year total average)
Monthly mean air temperature (ºC)			
April	10	8	11
May	17	16	17
June	22	21	22
July	22	23	24
August	22	23	23
September	17	21	18
October	11	11	11
Total precipitation (mm)			
April	121	148	98
May	108	180	136
June	225	26	122
July	73	26	115
August	148	30	130
September	138	30	85
October	119	64	65

Table 3
Leaf concentration of crude protein, nitrate, P, K, Mg, Ca, Mn, Fe, Cu, Zn, S for three pulse crops at three leaf removal rates at Curtiss farm, Ames - Iowa, 2013.

	Leaf CP g kg^{-1}	NO_3-N mg kg^{-1}	P mg kg^{-1}	K Mg kg^{-1}	Mg mg kg^{-1}	Ca mg kg^{-1}	Mn mg kg^{-1}	Fe mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}	S mg kg^{-1}
Leaf removal											
33	256	694	4116	24134	5264	22517b	94	155	18	128	3550
66	251	515	4057	22758	5861	25334ab	106	293	19	136	3659
99	242	553	4198	24693	5706	26871a	128	210	19	151	3702
Crop											
Soybean	267a	690a	3752c	24430	6226a	16669c	74b	271	17	153	3231c
Iron and Clay	247b	762a	4094b	23853	6144a	31409a	176a	142	16	142	3523b
Lablab	236b	311b	4524a	23302	4461b	26644b	78b	246	22	121	4158a
Significance						*P* > F					
Leafing (L)	ns	ns	ns	ns	ns	*	Ns	ns	ns	ns	ns
Crop (C)	**	***	***	ns	***	***	***	ns	ns	ns	***
L × C	ns	ns	ns	ns	ns	Ns	Ns	ns	ns	ns	ns

*Significant at P≤ 0.05
**Significant at P≤0.01
***Significant at P≤0.001
CP- Crude protein

Table 4
Leaf contents, crude protein(CP), nitrate, P, K, Mg, Ca, Mn, Fe, Cu, Zn and S from three pulse crops with three leaf removal rates at Curtiss farm, Ames - Iowa, 2013.

	P mg m^{-2}	K mg m^{-2}	Mg mg m^{-2}	Ca mg m^{-2}	Mn mg m^{-2}	Fe mg m^{-2}	Cu Mg m^{-2}	Zn mg m^{-2}	S mg m^{-2}	Nitrate mg m^{-2}
Leaf removal										
33	64 b	384 b	84 b	346 b	1.3 b	2.6	0.28	2.1	56 b	10
66	101 ab	560 ab	145 a	621 ab	2.6 ab	7.4	0.47	3.1	91 ab	13
99	130 a	770 a	170 a	826 a	3.7 a	6.7	0.55	4.4	115 a	15
Crop										
Soybean	94 ab	582	158	427 b	1.9	6.6	0.41	3.6	81 ab	17 a
Iron and Clay	79 b	468	119	622 ab	3.4	2.7	0.30	2.9	68 b	14 a
Lablab	122 a	663	123	746 a	2.2	7.4	0.59	3.1	112 a	7 b
Significance						*P* > F				
Leafing (L)	**	**	**	**	**	ns	Ns	ns	**	Ns
Crop (C)	*	Ns	ns	*	ns	ns	Ns	ns	*	**
L × C	ns	Ns	ns	ns	ns	ns	Ns	ns	ns	Ns

*Significant at P≤ 0.05.
**Significant at P≤0.01
***Significant at P≤0.001

Table 5
Stand density, biomass and yield components for soybean, lablab and Iron and Clay at Curtiss farm, Ames - Iowa, 2013.

	Stand V4	Stand R8	Biomass	Yield	Pods	Seed	Seed	Seed	Dry leaves	Soybean height R8
	no. m^{-2}	no. m^{-2}	kg ha^{-1}	kg ha^{-1}	no. m^{-2}	no. pod^{-1}	no. m^{-2}	mg $seed^{-1}$	kg ha^{-1}	Cm
Leafing										
0	27.0	26.8	4483	1203	452	2.6	728	118	0	88
33	27.0	29.5	4844	1233	483	3.3	771	121	157 b	83
66	28.2	24.4	4402	946	395	4.0	588	123	247 ab	82
99	28.5	28.0	4089	1019	448	0.9	656	89	307 a	75
Crop										
Soybean	20 c	20.4 b	4839	2182 a	880 a	1.5 b	1350 a	161 a	250	.
Iron and Clay	37 a	30.0 a	3902	19 b	10 b	4.0 a	21 b	65 b	193	.
Lablab	26 b	31.5 a	4623	0	0	0	0	0	268	.
Significance				*P* > F						
Leafing (L)	Ns	ns	ns	ns	Ns	Ns	Ns	Ns	**	ns
Crop (C)	***	**	ns	***	***	*	***	***	Ns	.
L × C	Ns	ns	**	ns	Ns	Ns	Ns	Ns	Ns	.

*Significant at P≤ 0.05
**Significant at P≤0.01
***Significant at P≤0.001

Table 6

Neutral and acid detergent fiber, acid detergent lignin, hemicellulose, cellulose, and ash concentrations of leaves for three pulse crops at Curtiss farm, Ames - Iowa, 2013.

	NDF g kg^{-1}	ADF g kg^{-1}	ADL g kg^{-1}	Hemicellulose g kg^{-1}	Cellulose g kg^{-1}	Ash g kg^{-1}
Leafing						
33	413	316	64.8	97.6	251	5.2
66	406	308	64.9	96.8	243	4.9
99	404	308	64.5	97.9	244	5.3
Crop						
Soybean	425 a	327 a	71 a	98 a	256 a	5.7
Iron and clay	383 b	292 b	56 b	91 b	235 b	4.9
Lablab	417 a	313 a	67 a	103 a	246 ab	4.9
Significance			*P* > F			
Leafing (L)	ns	ns	ns	ns	ns	ns
Crop (C)	***	**	***	***	*	ns
L × C	ns	ns	ns	ns	ns	ns

*Significant at P≤ 0.05.
**Significant at P≤0.01
***Significant at P≤0.001

Table 7
Leaf concentration, crude protein(CP), nitrate, P, K, Mg, Ca, Mn, Fe, Cu, Zn and S from four pulse crops at three leaf removal rates at Agronomy farm, Ames - Iowa, 2014.

	Leaf CP	NO3-N	P	K	Mg	Ca	Mn	Fe	Cu	Zn	S
	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}`	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Leaf removal											
0	-	-	-	-	-	-	-	-	-	-	-
33	223	291	3889	21789	3705	10748	83 b	104 b	10	56	2380
66	213	282	3332	21285	3501	12175	107 b	184 b	9	53	2233
99	203	206	2936	18691	3591	14653	156 a	297 a	9	29	2083
Crop											
Soybean	234	288 ab	2781	22467	4505 a	10636 b	81 b	165 b	8	32	2159
CA46	243	434 a	3432	22025	3999 a	12068 ab	163 a	189 ab	10	44	2211
Top crop	207	185 ab	3411	20037	3667 ab	15604 a	138 a	141 b	10	59	2203
Lablab	169	131 b	3919	17824	2225 b	11793 ab	80 b	284 a	9	48	2159
Significance					*P* > F						
Leafing (L)	ns	ns	ns	ns	ns	ns	**	***	ns	ns	ns
Crop (C)	ns	*	ns	ns	**	*	***	**	ns	ns	ns
L × C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*Significant at P≤ 0.05.
**Significant at P≤0.01
***Significant at P≤0.001

Table 8

Leaf content of Nitrate, P, K, Mg, Ca, Mn, Fe, Cu, Zn, S for three pulse crops at three leaf removal rates at Agronomy farm, Ames - Iowa, 2014.

	NO3-N mg m^{-2}	P mg m^{-2}	K mg m^{-2}	Mg mg m^{-2}	Ca mg m^{-2}	Mn mg m^{-2}	Fe mg m^{-2}	Cu mg m^{-2}	Zn mg m^{-2}	S mg m^{-2}
Leaf removal										
0	-	-	-	-	-	-	-	-	-	-
33	6 b	102b	536 b	80 b	254 c	2 c	3 c	0.24 b	1.4 b	58 b
66	16 a	210 a	1277 a	196 a	725 b	6 b	12 b	0.52 a	3.6 a	137 a
99	11 ab	204 a	1189 a	197 a	890 a	9 a	19 a	0.58 a	2.1 ab	135 a
Crop										
Soybean	12 ab	145 c	1036 b	189 a	526 b	4 c	10	0.39 b	1.7	107 ab
CA46	21 a	208 a	1271 a	2031 a	710 a	9 a	13	0.59 a	2.5	131 a
Top Crop	9 ab	184 ab	1030 b	163 a	794 a	7 b	9	0.47 ab	3.4	115 ab
Lablab	44b	151 bc	667 c	76 b	463 b	3 c	13	0.33 b	1.9	88 b
Significance					*P* > F					
Leafing (L)	*	***	***	***	***	***	***	***	**	***
Crop (C)	**	***	***	***	***	**	ns	**	ns	*
L × C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*Significant at P≤ 0.05.
**Significant at P≤0.01
***Significant at P≤0.001

Table 9

Stand density, biomass and yield components for soybean, lablab, CA46 and Top Crop at Agronomy farm, Ames - Iowa, 2014.

	Stand V4	Stand R8	Biomass	Yield	Pods	Seed	Seed	Seed	Seed CP	Leaf yield
	no. m^{-2}	no. m^{-2}	kg ha^{-1}	kg ha^{-1}	no. m^{-2}	no. pod^{-1}	no. m^{-2}	mg $seed^{-1}$	g kg^{-1}	kg ha^{-1}
Leaf removal										
0	37	38	5007	2375 a	332	4.8	1439 a	160 a	292	0
33	36	35	4111	1909 ab	410	3.8	1190 ba	151 ab	286	122 b
66	36	37	4896	1616 b	344	4.2	1042 b	143 b	284	435 a
99	36	35	4737	1548 b	317	4.6	1036 b	141 b	296	451 a
Crop										
Soybean	32 b	42	6673 a	3411 a	743 a	2.7 b	1943 a	178 a	353 a	325 ab
CA46	38 a	34	4847 ab	1220 b	151 b	5.8 a	857 b	141 b	266 b	422 a
Top Crop	41 a	33	3646 b	955 b	158 b	4.7 a	731 b	127 c	248 c	359 a
Lablab	33 b	-	3586 b	0	0	0	0	0	0	239 b
Significance					*P* > F					
Leafing (L)	ns	ns	ns	**	ns	ns	**	**	ns	***
Crop (C)	ns	ns	**	***	***	***	***	***	***	*
L × C	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*Significant at P≤ 0.05.
**Significant at P≤0.01
***Significant at P≤0.001

Table 10
Dry matter, neutral and acid detergent fiber, acid detergent lignin, hemicellulose, cellulose, and ash concentrations from leaves of four pulse crops at Agronomy farm, Ames - Iowa, 2014.

	NDF	ADF	ADL	Hemicellulose	Cellulose	Ash
	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}
Leaf removal						
0	-	-	-	-	-	-
33	201	153	26 b	48	127	0.3 b
66	237	177	35 a	60	143	3.0 a
99	217	159	34 ab	58	125	4.8 a
Crop						
Soybean	264	202 a	37 a	61	165 a	2.2 b
CA46	208	151 b	28 b	57	124 b	1.6 b
Top Crop	207	151 b	28 ab	56	123 b	1.1 b
Lablab	195	148 c	33 ab	47	115 c	6.0 a
Significance		*P* >F				
Leafing (L)	ns	ns	ns	ns	ns	**
Crop (C)	ns	*	ns	ns	*	***
L × C	ns	ns	ns	ns	ns	ns

*Significant at P≤ 0.05
**Significant at P≤0.01
***Significant at P≤0.001

Table 11

Regression equations for average leaf concentration of Ca, Fe and Mn plotted against leaf removal rate for 2013 and 2014.

Average Ca concentration = 6895.5x + 20301,	$r^2 = 0.9632$	(2013)
Average Fe concentration = 2.86x + 55.9,	$r^2 = 0.9269$	(2014)
Average Mn concentration = 0.953x + 57.4,	$r^2 = 0.9334$	(2014)

Regression was only done for parameters that were significantly affected by leaf removal rate/percentage

Table 12

Regression equations for average yield (kg ha^{-1}), seed number m^{-2} and seed weight (mg) plotted against leaf removal rate, Agronomy, 2014.

Average yield =	-732.69x + 2116.3,	$r^2 = 0.9517$
Average seed number =	-369.77x + 1296.2,	$r^2 = 0.9185$
Average seed weight =	-16.779x + 154.91,	$r^2 = 0.9914$

Regression was only done for parameters that were significantly affected by leaf removal rate/percentage.